INTERACTIONS BETWEEN THE NORADRENERGIC AND DOPAMINERGIC NEUROTRANSMITTER SYSTEMS IN THE RAT BRAIN

by

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DECLARATION

I the undersigned declare that the work contained in the dissertation is my own original work and has not previously in its entirety or in any part been submitted at any University for a degree

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DATE 8. 8. 1990

SUMMARY

The development of a dissection technique enabled the nucleus accumbens to be dissected into six and the striatum into eighteen discrete areas. The concentration of monoamines in these areas was analysed by high performance liquid chromatography (HPLC) with electrochemical detection.

The distribution of the different monoamines in the nucleus accumbens was not identical. In general, concentrations were found to be low in the rostral area of the nucleus accumbens. Marked differences were observed in the medial area. Dopamine (DA) levels were significantly lower in the ventrorostral than in the dorsorostral nucleus accumbens and high in both medial and caudal areas. Noradrenaline (NA) and serotonin (5-HT) levels were considerably lower than those of DA. The NA concentration was highest in the caudal area of the nucleus accumbens and the 5-HT concentration was highest in the ventrocaudal area. There was evidence for a rostrocaudal decrease in DA and 5-HT turnover in the nucleus accumbens. In the striatum, DA levels were higher rostrally than caudally, the lowest levels being found in the globus pallidus. NA levels were low throughout the striatum but significantly higher in the globus pallidus. 5-HT and 5-hydroxyindole acetic acid (5-HIAA) levels were higher ventrally than dorsally and increased along the rostrocaudal axis.

Selective lesioning of the locus coeruleus (LC) noradrenergic neurons by the administration of N-(2-chloroethyl)-N-ethyl-2- bromobenzylamine hydrochloride (DSP4) or by direct infusion of 6-hydroxydopamine (6-OHDA) resulted in a decrease in NA concentration in the rostral area of the nucleus accumbens. DA and 5-HT levels were not affected by these lesions. DSP4 lesions caused increased 3,4-dihydroxyphenyl acetic acid (DOPAC) turnover in the ventromedial and ventrocaudal areas, indicating increased catechol-O-methyltransferase (COMT) activity in these areas. 6-OHDA lesions of the medial forebrain bundle (MFB) resulted in decreased NA and DA levels in all areas of the nucleus accumbens. DA turnover was increased, indicating increased monoamine oxidase (MAO) activity in th medial and caudal areas after MFB lesions.

Increased 5-HIAA/5-HT ratios were also found in the medial and caudal areas.

The distribution of DA D1 and D2 receptors in the nucleus accumbens was determined by means of radioligand binding assays. [^{3}H]SCH23390 was used to label DA D1 receptors and [^{3}H]spiperone was used for DA D2 receptors. The distribution of DA D1 and D2 receptors was not superimposable although there was considerable overlap. DA D1 receptor density roughly followed the DA innervation, being low rostrally and high medially and caudally. There were no dorsoventral differences. In the ventrorostral area it appears that relatively few, more active neurons can activate a similar number of postsynaptic DA D1 receptors. DA D2 receptor density was lowest in the ventrorostral area, highest in the dorsomedial area and similar in the remaining areas of the nucleus accumbens.

Chronic treatment with desipramine resulted in no significant changes in DA D1 or D2 receptor number or affinity in the nucleus accumbens, therefore increased dopaminergic transmission occurring after chronic antidepressant treatment would appear not to be due to direct changes in DA receptor binding.

OPSOMMING

'n Disseksietegniek is ontwikkel waardeur die nucleus accumbens in ses en die striatum in agtien diskrete areas gedissekteer kon word. Monoamienkonsentrasies in hierdie areas is deur middel van hoë werkverrigtings-vloeistofchromatografie met elektrochemiese deteksie geanaliseer.

Die verspreiding van die verskillende monoamienes in die nucleus accumbens was oneweredig. Oor die algemeen is 'n lae monoamienkonsentrasie in die rostrale gedeelte waargeneem. Duidelike verskille is in die mediale gedeeltes gemeet. Dopamien(DA)- vlakke was betekenisvol laer in die ventrorostrale area van die nucleus accumbens as in die dorsorostrale area, terwyl hoër vlakke in beide die mediale en kaudale areas waargeneem is. Die vlakke van noradrenalien (NA) en serotonien (5-HT) was heelwat laer as die vlakke van DA. Die NA-konsentrasie was die hoogste in die kaudale area van die nucleus accumbens en die 5-HT-vlakke die hoogste in die ventrokaudale area. Dit wil voorkom asof die omset van beide DA and 5-HT in die rostrale gedeelte van die nucleus accumbens laer is as in die kaudale gedeelte. In die striatum is gevind dat DA-vlakke rostraal hoër is as kaudaal met die laagste vlakke in die globus pallidus. NA-vlakke was laag in die striatum maar betekenisvol hoër in die globus pallidus. 5-HT en 5-hidroksieindoolasynsuur(5-HIAA)-vlakke was hoër in die ventrale dele as in die dorsale dele en is verhoog langs die rostrokaudale as.

Selektiewe beletseling van die locus coeruleus (LC) noradrenerge neurone deur toediennig van N-(2-chloroethiel)-N-etiel-2- bromobenzylamien hidrochloried (DSP4) of deur direkte inspuiting van 6-hidroksiedopamien (6-OHDA), het 'n verlaging in die NA-konsentrasie van die rostrale nucleus accumbens area veroorsaak. DA- en 5-HT-vlakke is nie deur hierdie beletseling beïnvloed nie. DSP4-beletseling het 'n verhoging in die 3,4-dihidroksie-fenielasynsuur (DOPAC)-omset in die ventromediale en ventrokaudale areas veroorsaak wat dui op 'n verhoogde katesjol-O-metiel-transferase (COMT) aktiwiteit in hierdie areas. 6-OHDA beletsing van die mediale voorbreinbondel (MVB) het 'n verlaging in die vlakke van NA en DA

in al die areas van die nucleus accumbens veroorsaak. Die DA-omset was verhoog en dui op 'n verhoging in die aktiviteit van monoamien-oksidase (MAO) in die mediale en kaudale areas na die beletseling van die MVB. Ook is 'n vehoging in die 5-HIAA/5-HT verhouding in die mediale en kaudale areas waargeneem.

Die verspreiding van DA D1- en D2-reseptore in die nucleus accumbens is bepaal deur middel van radioligand-bindingsmetodes. [^{3}H]SCH23390 is gebruik om die DA D1-reseptor te merk en [^{3}H]spiperoon om die DA D2-reseptor te merk. Die verspreiding van DA D1- en D2-reseptore was nie identies nie alhoewel daar 'n groot mate van oorvleueling was. Die DA D1-reseptordigtheid het rofweg ooreengekom met DA innervering en was dus laag rostraal en hoog mediaal en kaudaal. Daar was geen dorsoventrale verskille nie. Dit wou voorkom asof relatief minder, maar meer aktiewe neurone in die ventrorostale gedeelte voorkom wat 'n ooreenkomstige aantal postsinaptiese DA D1-reseptore kan aktiveer. DA D2-reseptordigtheid was die laagste in die ventrorostrale gedeelte en die hoogste in die dorsomediale area van die nucleus accumbens, maar het nie in die oorblywende areas verskil nie.

Kroniese behandeling van rotte met desipramien het geen veranderings in die DA D1- en D2-reseptorgetal of affiniteit in die nucleus accumbens tot gevolg gehad nie. Dit wil dus voorkom asof die verhoging in dopaminerge transmissie na kroniese behandeling met antidepressiewe middels nie die direkte gevolg van 'n verandering in DA reseptorbinding is nie.

In this research project the statistical planning, analysis and subsequent recommendations were performed in consultation with the Institute for Biostatistics of the Medical Research Council.

TO MY HUSBAND

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INTRODUCTION

Several hypotheses have been put forward in order to explain the aetiology of depression. Dysregulation of the noradrenergic system, a relative deficiency of noradrenaline (NA) at functionally active sites in the brain, or serotonergic hypoactivity have been proposed to occur in depression. The role of dopamine in depression has also been implicated by various studies. For example, cerebrospinal fluid (CSF) levels of dopamine (DA) metabolites, 3,4-dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) have been reported to be lower in depressed patients. Another hypothesis for the aetiology of depression proposes that in depression there may be hypoactivity of a reward system in the brain in which mesolimbic dopaminergic neurons are involved. There has therefore been increasing interest in the nucleus accumbens which is a major terminal area for these dopaminergic neurons which arise in the ventral tegmental area (VTA) of the midbrain. There has been histological evidence that the nucleus accumbens is not a homogeneously organized structure, and interactions between monoaminergic neurotransmitters have been suggested to occur in this area. A detailed investigation of the distribution of monoamines in different areas of the nucleus accumbens was therefore carried out and compared to those in the striatum, an area which receives dense dopaminergic innervation from the substantia nigra. Since changes in mesolimbic dopaminergic activity may be secondary to changes in noradrenergic and serotonergic systems, it was considered important to study local interactions between noradrenergic and other neurotransmitter systems in the nucleus accumbens. This was carried out by investigating the effect of noradrenergic denervation on the monoamines and their metabolites in different areas of the nucleus accumbens.

DA receptors have been classified into two major subtypes. DA D1 receptors which are positively coupled to adenylate cyclase and DA D2 receptors which are negatively coupled or uncoupled to this enzyme. Many studies have recently demonstrated the importance of interactions between DA D1 and D2 receptors in the brain, and local differences in receptor

distribution have been suggested by behavioural studies. The relative distribution of DA D1 and D2 receptors may be important in the expression of DA function therefore the distribution of these receptor subtypes in the nucleus accumbens was measured.

Behavioural studies have shown that chronic treatment with antidepressant drugs results in enhanced dopaminergic transmission. Various changes in DA receptor activity following antidepressant drug treatment have been suggested, particularly in the mesolimbic system. The effect of chronic treatment with the antidepressant drug, desipramine, on DA D1 and D2 receptors in the nucleus accumbens was therefore investigated so that further knowledge concerning the changes in DA receptor activity following antidepressant drug treatment may be obtained.

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ABBREVIATIONS

In addition to the conventional atomic symbolism and S.I. units, the following abbreviations are used throughout this thesis:

ACh	Acetylcholine
ANOVA	Analysis of variance
Bmax	Total binding capacity of saturable receptor
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
Ci	Curie
COMT	Catechol-O-methyl-transferase
CSF	Cerebrospinal fluid
DA	Dopamine
DOPAC	3,4-Dihydroxyphenyl acetic acid
dpm	Disintegrations per minute
DSP4	N-(2-Chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride
EDTA	Ethylenediamine tetra-acetic acid
EGTA	Ethylenebis(oxyethylenenitrilo)-tetraacetic acid
GABA	Gamma-amino butyric acid
^3H	Tritium
5-HIAA	5-Hydroxyindole acetic acid
HPLC	High performance liquid chromatography
HVA	Homovanillic acid
5-HT	Serotonin
IC_{50}	Concentration of inhibitor which causes 50% inhibition
i.p	Intraperitoneal
K_D	Equilibrium dissociation constant

K_i	Inhibition constant
LC	Locus coeruleus
MAO	Monoamine oxidase
MFB	Medial forebrain bundle
MHPG	3-methoxy-4-hydroxyphenylglycol
3-MT	3-methoxy-4-hydroxyphenylethylamine
NA	Noradrenaline
6-OHDA	6-Hydroxydopamine
SEM	Standard error of the mean
VTA	Ventral tegmental area

CHAPTER 1

MONOAMINE NEUROTRANSMITTER SYSTEMS IN THE BRAIN

1.1 MONOAMINE NEUROTRANSMITTERS

1.1.1 Introduction

The morphology of monoaminergic neurons has been well characterized in the central nervous system (Brodal, 1981; Triggle and Triggle, 1976). Cell bodies, which have a large number of receptive dendrites projecting from them, give rise to long axons along which impulses arising in the neuron are transmitted to dense networks of nerve terminals. Varicosities occur along the nerve axons (Oades and Halliday, 1987, Lindvall and Stenevi, 1978; Jacobowitz, 1978) which, like nerve terminals, synthesize and release neurotransmitters in response to nerve impulses. The specialized areas of contact where transmission occurs are called synapses and are mainly axodendritic, but axosomatic and axoaxonic synapses also occur (Brodal, 1981). Release of neurotransmitters occurs when the membrane is depolarized by the nerve action potential Fluxes of calcium and potassium ions activate the storage vesicles which migrate to the cell boundary, fuse with the membrane and the neurotransmitter is released by exocytosis The neurotransmitter diffuses across the synaptic cleft to activate receptors on postsynaptic neurons (Fig 1.1) which leads to excitatory or inhibitory responses that are activated by passive ionic conductances (Bloom, 1988). The action of neurotransmitters can be terminated by cellular uptake mechanisms or by enzymatic inactivation.

The catecholamines noradrenaline (NA) and dopamine (DA), and the indoleamine serotonin (5-HT) are important monoamine neurotransmitters in the brain. NA is the transmitter in certain tracts of the brain such as the h pothalamus and the cerebral and cerebellar cortices, while DA is the major neurotransmitter in the basal ganglia and limbic system. 5-HT is also present in the limbic system as well as in other areas of the brain.

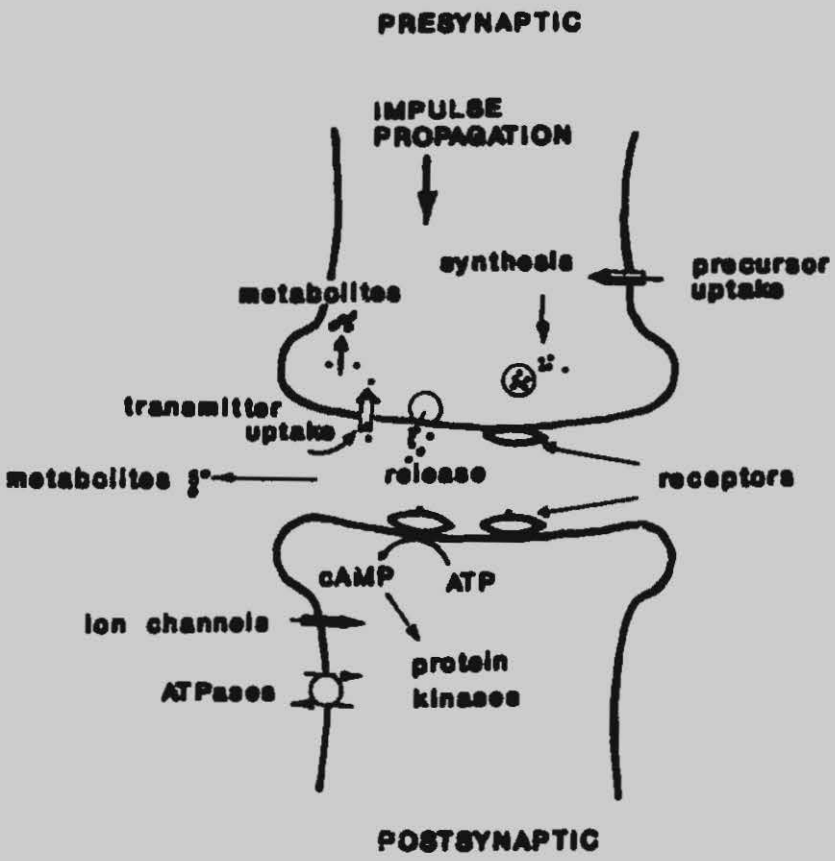

Fig. 1.1
Schematic representation of a synaptic junction.

1.1.2 Catecholamines

Synthesis of NA and DA takes place in the cytoplasm of catecholaminergic nerve terminals The amino acid tyrosine is taken up into the nerve ending where it is hydroxylated to L-dopa by tyrosine hydroxylase. L-dopa is then converted to DA by dopa decarboxylase (Fig 1.2). DA is subsequently transported from the cytoplasm to vesicles where it is stored in dopaminergic neurons. In noradrenergic neurons it is converted to NA by dopamine β-hydroxylase (Fig 1.2). In certain parts of the brain NA is further methylated to form adrenaline by the cytoplasmic enzyme phenylmethanolamine N-methyltransferase using S-adenosylmethionine as the methyl donor (Tri and Triggle, 1976). Tyrosine hydroxylase is the rate limiting enzyme in the pathway and the synthesis rate of catecholamines or L-dopa can therefore be used as an index of tyrosine hydroxylase activity (Wolf et al., 1986)

Catecholamines are stored in high concentration in vesicles either free or as a complex with adenosine triphosphate. They are also found free in the cytoplasmic fluid (Triggle and Triggle, 1976), therefore there are two mobile pools as well as the intravesicular reserve pool Catecholamines move by active uptake from the cytoplasmic mobile pool into the vesicles

The most important mechanism by which DA and NA may be removed from the synaptic cleft and their influence on receptors therefore terminated is by re-uptake. The neurotransmitters first cross the presynaptic membrane into the cytoplasm and then they are either metabolized or pass into the storage vesicles. Simple diffusion also accounts for some of the transmitter inactivation.

There are two important enzymes in the catabolism of catecholamines: monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). MAO is a mitochondrial enzyme which converts cytoplasmic catecholamines to the corresponding aldehyde by oxidative deamination (Triggle and Triggle, 1976). MAO has been classified into two functional forms, MAO-A and MAO-B based on substrate specificity. Deamination of DA in dopaminergic synaptosomes of

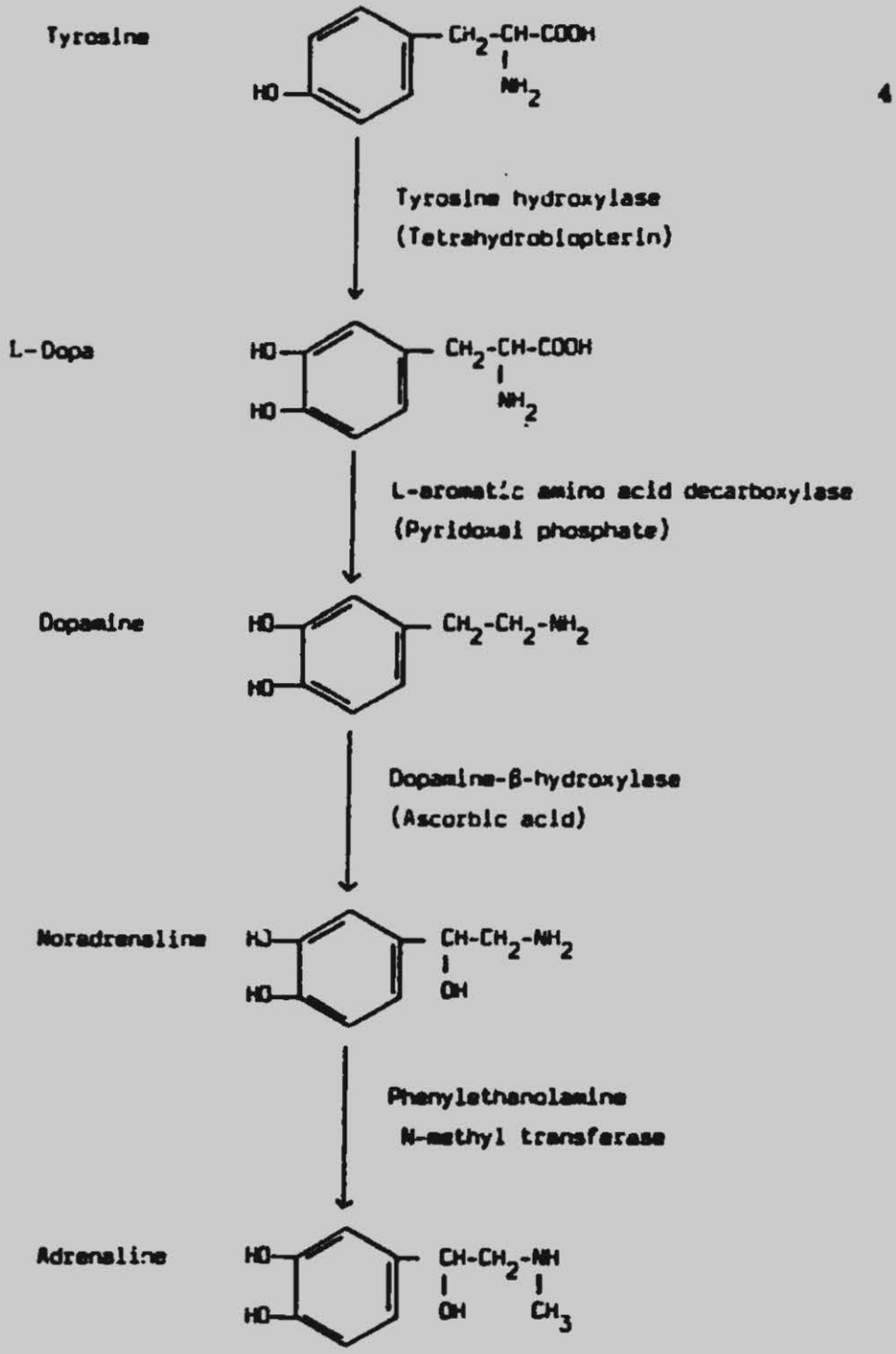

Fig. 1.2 Synthesis of catecholamines.

the striatum has been reported to be entirely due to MAO-A while, extraneuronally, 10% of the deamination of NA and DA was brought about by the B form and 90% by MAO-A (Fagervall et al., 1986). However, it has also been reported that substrates of MAO do not show absolute specificity and depend on the brain area and the concentration of enzyme type present (Koide et al., 1987). Methylation of DA or 3,4-dihydroxyphenyl acetic acid (DOPAC) occurs extraneuronally (Westerink, 1985) although COMT has also been suggested to occur intraneuronally (Vulto et al., 1986; Moore and Kelly,1978). COMT uses s-adenosylmethionine as a methyl donor to convert DA to 3-methoxy-4-hydroxyphenylethylamine (3-MT) (Fig. 1.3) and NA to normetanephrine (Fig 1.4). It changes one of the hydroxyl groups into a methoxy group. Further breakdown takes place involving MAO, COMT, aldehyde reductase and dehydrogenase (Figs. 1.3 and 1.4). DOPAC and homovanillic acid (HVA) are the only endogenous metabolites of DA formed in large amounts in the central nervous system; the 3-MT pathway is of minor importance with only 12% of the DA being converted to 3-MT whereas 88% is converted to DOPAC (Westerink, 1985). (Fig. 1.5). 3-Methoxy-4-hydroxyphenylglycol (MHPG) and 3,4-dihydroxyphenylglycol are the major metabolites of NA (Karege and Gaillard, 1986) (Fig. 1.4). Several mechanisms regulate the synthesis of catecholamines. End product inhibition has been reported to occur and tyrosine hydroxylase has also been suggested to be subject to feedback inhibition (Kilts et al., 1987; Chiodo, 1988). There is evidence that tyrosine hydroxylase activity can be controlled by presynaptic receptors (Moore and Kelly, 1978; Wolf et al., 1986; Imperato et al., 1988).

Because feedback mechanisms regulate the rate of DA synthesis, neurotransmission can be altered without altering the steady state concentration of transmitter (Altar et al., 1987). It has been reported that a significant proportion of DOPAC is derived from the metabolism of recently synthesized DA, and it was therefore suggested that short term changes in brain levels of DOPAC provide a useful index of alterations in the functional activity of DA neurons (Roth et al., 1976; Zetterström et al., 1988). The 3-MT pathway is a relatively minor route of DA metabolism, and is an indicator of DA release. It has therefore been suggested that DOPAC

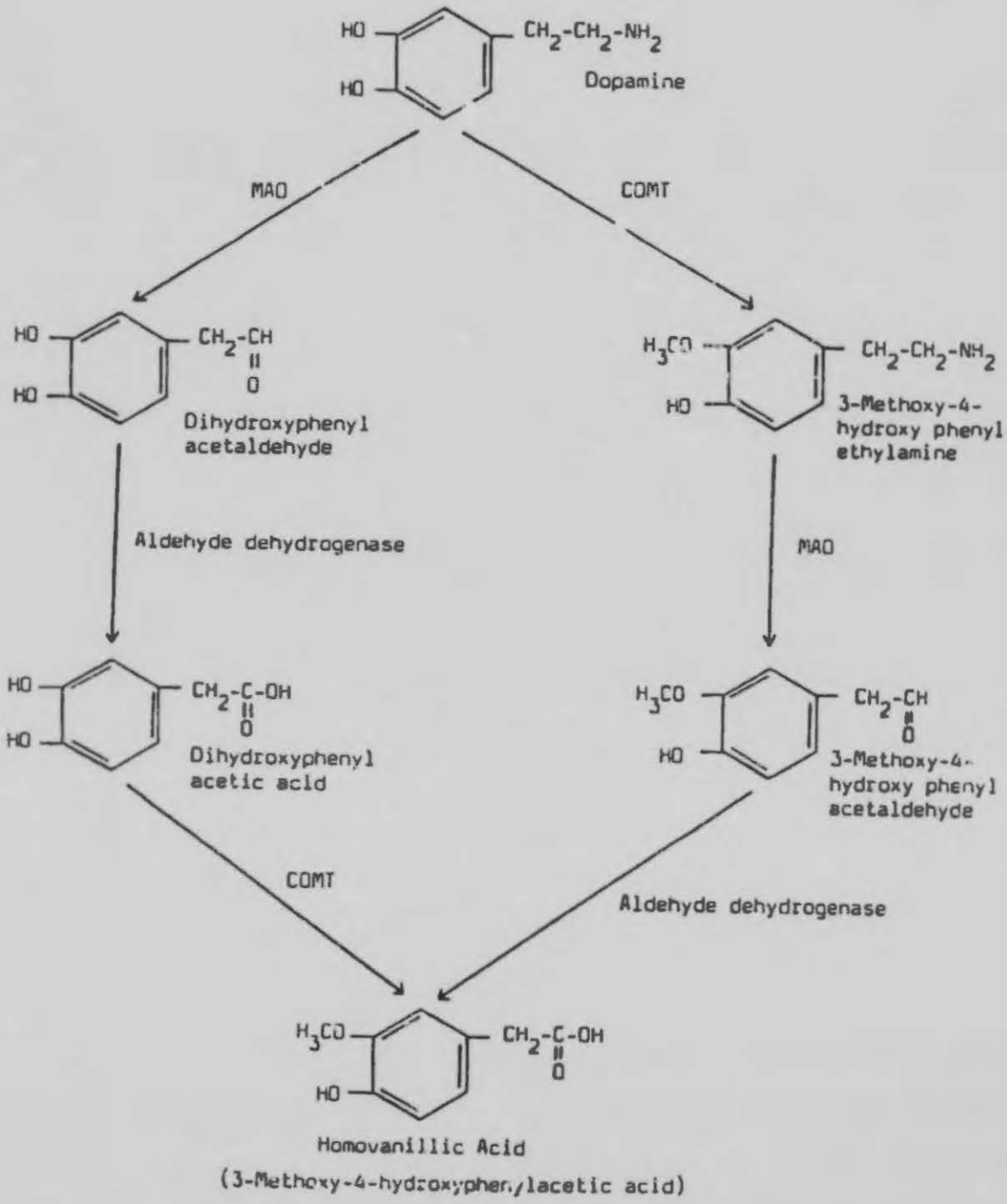

Fig. 1.3 Catabolism of dopamine.

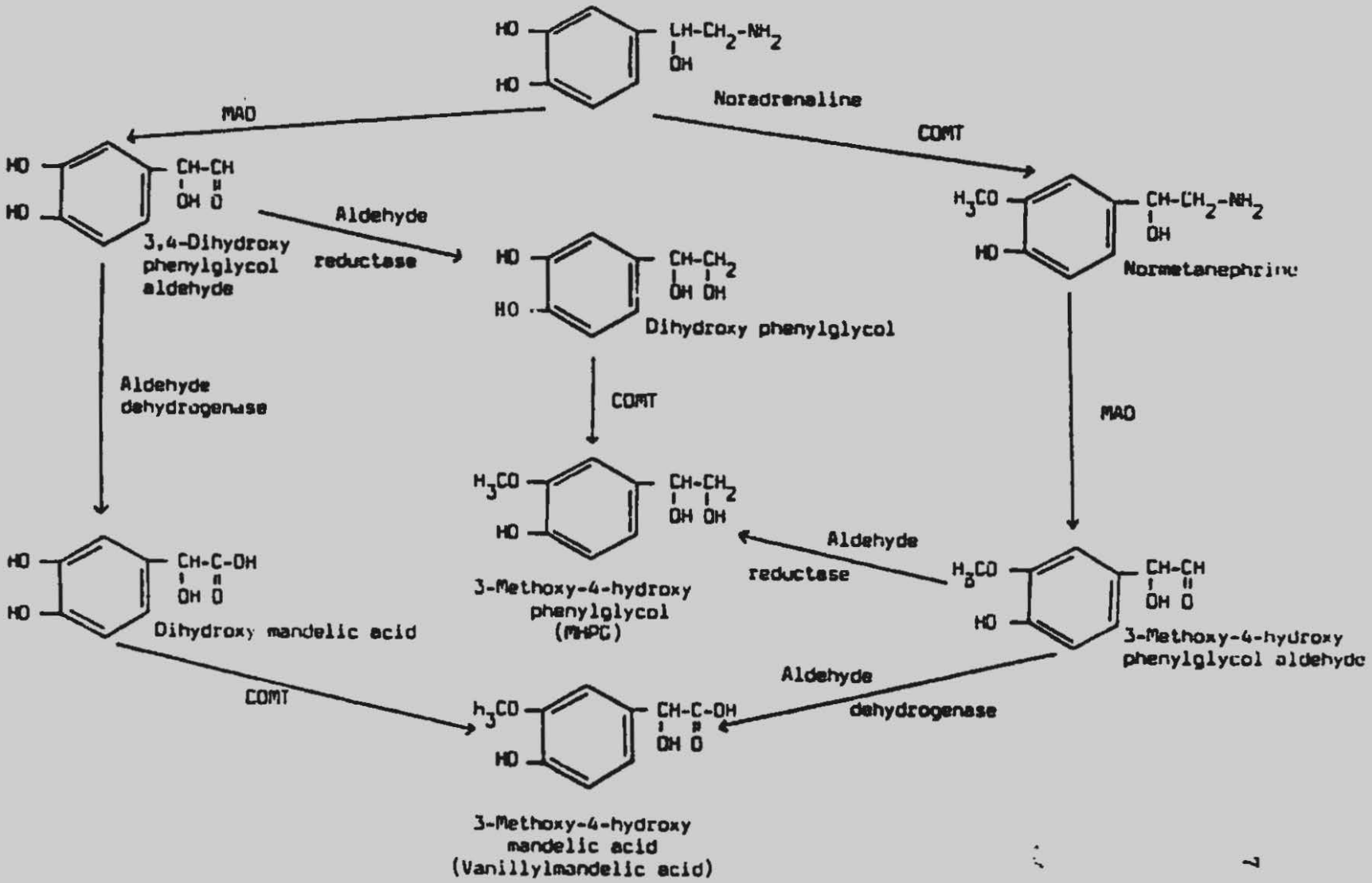

Fig. 1.4 Catabolism of noradrenaline.

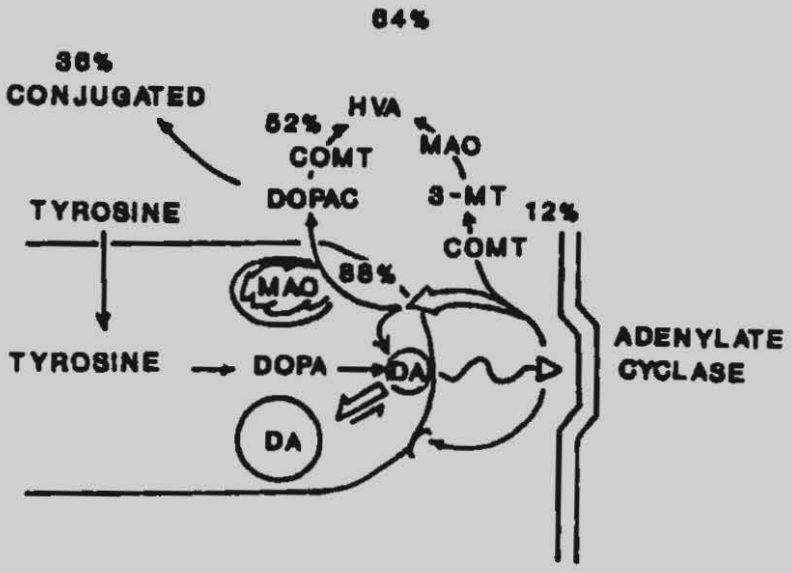

Fig. 1.5 Schematic diagram of a dopaminergic synapse indicating the percentages of metabolite, formed.

formation is a useful measure of DA turnover (Moore and Kelly, 1978) For this purpose metabolite/monoamine ratios have been widely used as a measure of DA turnover (Altar et al., 1987, Beal and Martin, 1985)

1.1.3 Indoleamines

The precursor of the indoleamine neurotransmitter, 5-HT, is the amino acid tryptophan Tryptophan hydroxylase catalyses the conversion of tryptophan to 5-hydroxytryptophan, which is then decarboxylated to form 5 HT (Fig 1 6) Like catecholamines 5 HT is actively transported from the cytoplasm into storage vesicles in the neuron terminals (Triggle and Triggle, 1976) and is stored in association with adenine nucleotides, mainly adenosine triphosphate 5-HT synthesis in the central nervous system depends on concentration of tissue tryptophan and the intrinsic activity of tryptophan hydroxylase (Hamon et al., 1981) In the 5 HT system end product inhibition does not appear to play a role in 5-HT synthesis, but negative feedback control of 5 HT synthesis by 5 HT is indicated by receptor mediated feedback (Kehr, 1985)

The release of 5-HT into the synaptic cleft is by ionic activation and exocytosis as described for catecholamines Re-uptake is the primary route of inactivation This process is energy dependent and can work against a considerable concentration gradient. Intracytoplasmic 5-HT can form a substrate for MAO, especially type A, although MAO-B contributes to approximately 20% of its deamination. (Fagervall and Ross, 1986) The enzyme converts 5-HT into 5 hydroxyindoleacetaldehyde, which is then oxidized by aldehyde dehydrogenase to the acidic metabolite 5 hydroxyindole acetic acid (5 HIAA) Under certain conditions 5 HIAA can be reduced to the alcoholic derivative 5 hydroxytryptophol (Fig 1 6)

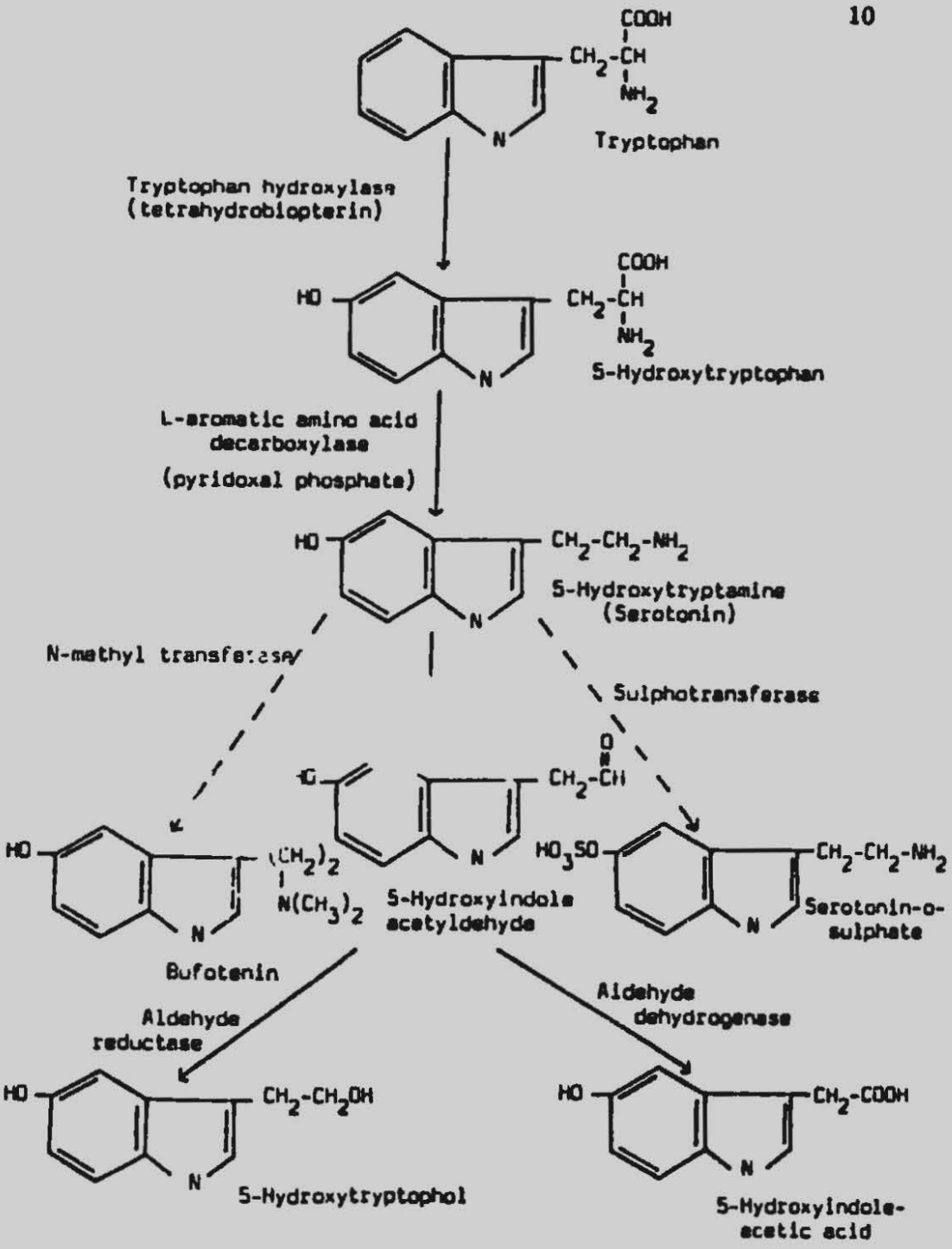

Fig. 1.8 Metabolism of serotonin.

1.2 MONOAMINE NEUROTRANSMITTER PATHWAYS

1.2.1 Noradrenergic pathways

The cell bodies of the noradrenergic neurons in the brain are mainly localized in the pons and medulla oblongata (Lindvall and Björklund, 1983; Olson and Fuxe, 1972). Two major ascending noradrenergic pathways have been described (Lindvall and Bjorklund, 1978, Ungerstedt, 1971a, Olson and Fuxe, 1972) The dorsal noradrenergic pathway, or dorsal tegmental bundle, and the ventral noradrenergic bundle (Olson and Fuxe, 1972, Lindvall and Stenevi, 1978, Jones and Moore, 1977). The dorsal noradrenergic pathway originates in the locus coeruleus (LC) of the pons (A6), an area containing the most abundant aggregation of noradrenergic cell bodies in the brain. A major projection ascends in a dorsal pathway traversing the midbrain tegmentum to the caudal diencephalon where the axons descend to join the medial forebrain bundle (MFB) at a caudal hypothalamic level (Fig 1.7) A smaller projection from the LC takes a ventral course through the midbrain tegmentum and enters the MFB via the mamillary peduncle and ventral tegmental area (VTA) (Jones and Moore, 1977, Lindvall and Bjorklund 1974) LC neurons are highly collateralized and ramify into terminals which have fine, spindle shaped varicosities (O'Donohue et al., 1979, Iversen,1984) There are widespread projections to the dorsal thalamus, but in the hypothalamus projections are restricted to the lateral hypothalamic area, periventricular nucleus, supraoptic nucleus and paraventricular nucleus (Nieuwenhuys et al., 1982). However, Lindvall and Björklund (1974), reported no significant LC innervation of the hypothalamus Further rostrally LC fibres traverse the zona incerta to terminate in the amygdaloid complex, neocortex, septal nuclei, olfactory bulb and a large component continues around the corpus callosum to terminate in the hippocampus (Jones and Moore, 1977) The caudate nucleus (Lindvall and Bjorklund, 1983) and nucleus accumbens have also been reported to be innervated by LC neurons (Swanson and Hartman, 1975).

The ventral noradrenergic pathway neurons have a more widespread origin with cell bodies in

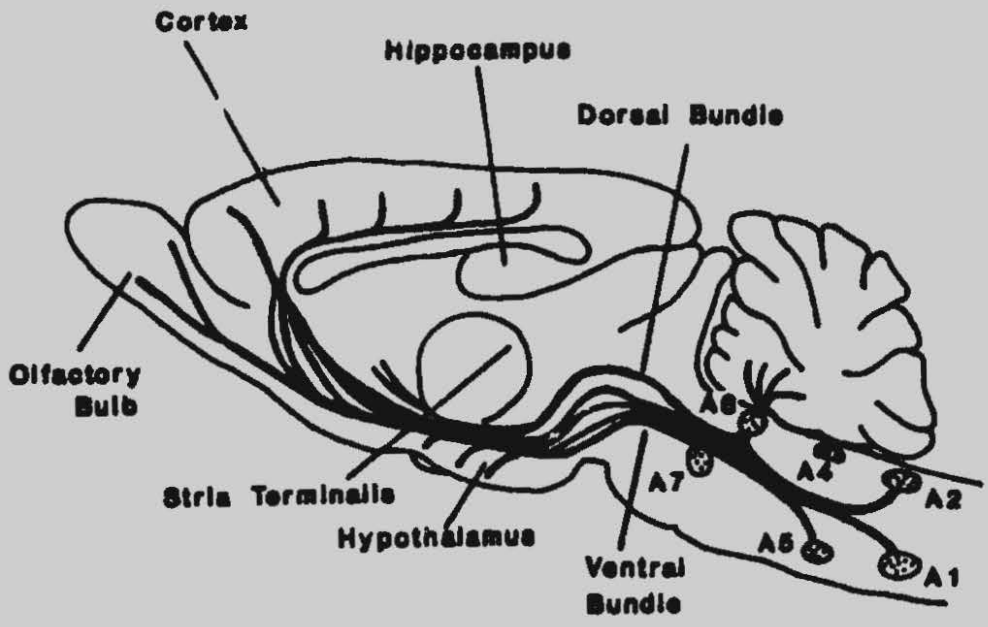

Fig. 1.7
Sagittal projection of the ascending noradrenergic pathways in the rat brain. The groups of cell bodies are depicted as dotted areas.

both the pons and medulla oblongata (Olson and Fuxe, 1972) Ascending projections from the so called A1 and A2 cell groups in the medulla are joined by neurons of the subcoeruleus nuclei (A5 and A7 according to the nomenclature of Dahlström and Fuxe, (1965)), (Fig. 1.7) which are located ventrolateral to the LC (A6). Neurons of the ventral noradrenergic bundle are coarser than LC neurons with spherical, closely spaced varicosities, and the two types of NA neurons can therefore be distinguished by fluorescence microscopy (Lindvall and Stenevi, 1978; Lindvall and Björklund, 1978). The ventral noradrenergic pathway divides into a network of "tegmental radiations" in the mesencephalon (Fig 1.7) before ascending dorsal to the substantia nigra to join the MFB. These neurons innervate the dorsomedial and ventromedial nuclei of the hypothalamus (Johnston et al., 1987), the thalamus, the preoptic, septal, amygdaloid nuclei and the piriform and neocortices. The neostriatum (Olson and Fuxe, 1972, Nieuwnhuys et al., 1982) and nucleus accumbens have also been reported to be innervated by ventral bundle neurons (Lindvall and Björklund, 1974, O'Donohue et al., 1979) A descending noradrenergic pathway has also been described (Lindvall and Björklund, 1974, Olson and Fuxe, 1972)

1.2.2 Dopaminergic pathways

DA cell bodies in the midbrain are located in the pars compacta of the substantia nigra (A8 and A9) of the rat brain (Fig 1.8). These cell bodies merge with those of the VTA (A10). Neurons from the substantia nigra ascend in the MFB in a dorsolateral position forming the nigrostriatal pathway which runs through the crus cerebri and internal capsule to innervate the caudate nucleus, putamen, globus pallidus and amygdala There appears to be a topographical arrangement of neurons in the nigrostriatal pathway, the dorsal and lateral fibres innervating the caudal regions and the ventral fibres terminating in rostral and ventral areas (Altar and Hauser, 1987, Iversen, 1984) This pattern extends to the neurons of the VTA as laterally situated VTA neurons project to the most medial and ventral parts of the caudate

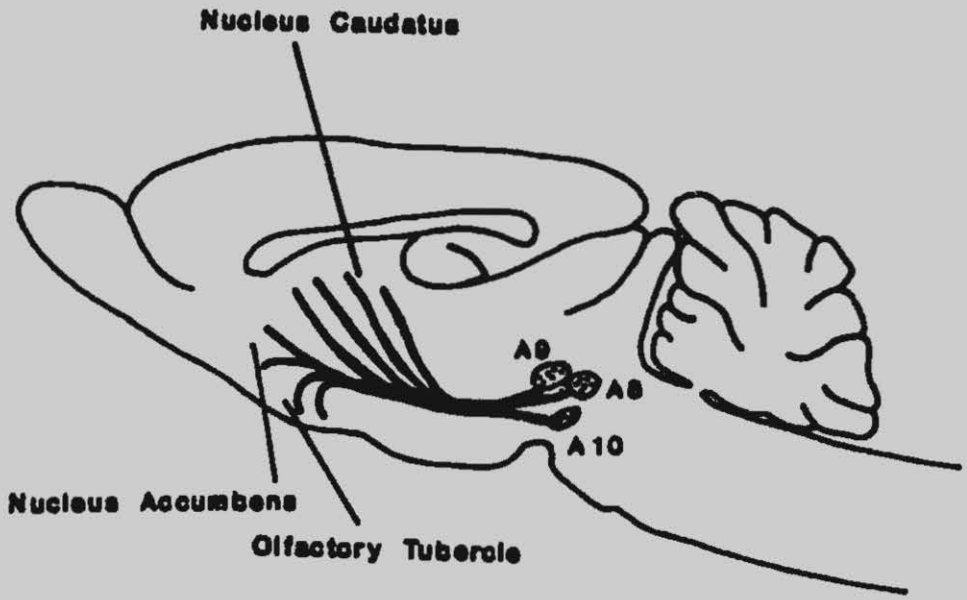

Fig. 1.8
Sagittal projection of the ascending dopaminergic pathways in the rat brain. The groups of cell bodies are depicted as dotted areas.

putamen (Lindvall and Björklund, 1978; Oades and Halliday, 1987). DA neurons arising in the VTA (A10) form the mesolimbic pathway which runs ventromedially to the nigrostriatal neurons in the MFB. Most of these neurons, arising in the VTA, terminate in the nucleus accumbens, bed nucleus of the stria terminalis, olfactory system, and septal nuclei. Rostrally fibres continue to the frontal, cingulate and entorhinal cortices (Oades and Halliday, 1987, Lindvall and Björklund, 1983). The hypothalamus, thalamus and hippocampus also receive dopaminergic innervation from neurons arising in the VTA (Oades and Halliday1987).

1.2.3 Serotonergic pathways

The serotonergic pathways emanate from a number of cell bodies which have been designated B1 to B9 situated mainly in the midline raphe nuclei although a number of cells are located lateral to the raphe nuclei (B3, B6, and B9),(Fig 1.9). Two rostral projections, the dorsal raphe forebrain tract and the median raphe forebrain tract innervate the forebrain diffusely, via the MFB (Nieuwenhuys et al., 1982). In particular the B5 and B6 cell bodies in the pons have been reported to project to the forebrain (Jacobowitz, 1978). Neurons from the midbrain B7 (dorsal raphe) area pass through the substantia nigra to the caudal tip of the caudate, the amygdala nuclei and piriform cortex. Fibres then extend through the subiculum to the molecular layer of the dentate gyrus in the hippocampus. Parts of the thalamus and hypothalamus also receive innervation from the dorsal raphe and further rostrally dorsal raphe neurons terminate in the nucleus accumbens and olfactory system (Azmitia and Segal, 1978. Iversen,1984). Median raphe (B8) neurons have also been suggested to innervate a number of areas in the forebrain with serotonergic fibres (Azmitia and Segal, 1978). Four additional tracts of serotonergic fibres have been reported to lie outside the MFB. These are the dorsal raphe arcuate tract, dorsal raphe periventricular tract, dorsal raphe cortical tract and raphe medial tract.

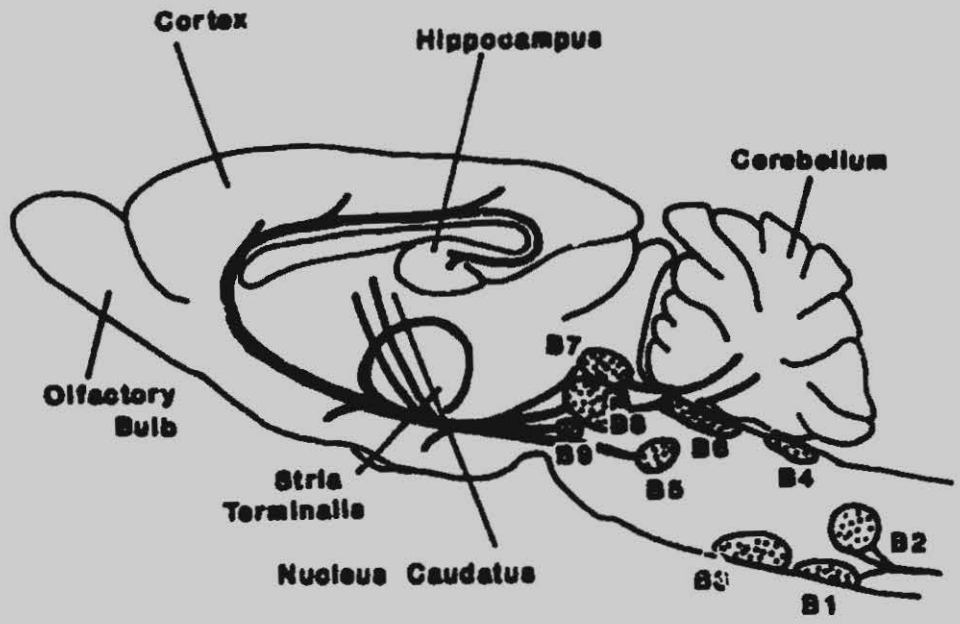

Fig. 1.9
Sagittal projection of the ascending seroton-.gic pathways in the rat brain. The groups of cell bodies are depicted as dotted areas.

1.2.4 Lesions of neurotransmitter pathways

In order to investigate different neuron connections or interactions between neurotransmitter systems, neuron pathways may be lesioned and the effects on various parameters in terminal areas may be studied Neurotransmitter pathways may be lesioned electrolytically (Plaznik et al., 1982, Stachowiak et al., 1987), by cutting the neurons (Brodel, 1981, Arnt and Perregaard, 1987), or chemically using a variety of neurotoxins Kainic acid, quinolinic acid and ibotenic acid are neurotoxins which exert their effect on sensitive neurons (via glutamate and aspartate receptors) by a strong excitation When sufficiently high doses are used the neurons become irreversibly depolarized The greatest effect is to the cell body while the axon has been reported to be relatively spared (Contestabile et al., 1984, Barone et al., 1987). 6-Hydroxydopamine (6-OHDA) is known to have neurodegenerative actions on catecholaminergic neurons (Breese and Cooper, 1977, Jonsson, 1980, Berger et al., 1988), while N-(2-chloroethyl)-N ethyl-2-bromobenzylamine hydrochloride (DSP4) has been reported to be a selective noradrenergic neurotoxin (Jonsson et al., 1981, Lookingland et al., 1986) 6-OHDA is taken up and accumulated by neurons that have a membrane transport mechanism for catecholamines and toxic effects occur when a certain critical concentration in the extragranular cytoplasm has been reached (Jonsson, 1980). The neurotoxic action of 6-OHDA is rapid (half to one hour after administration) but it must be injected directly into the ventricle, the neuron pathway or brain tissue as it does not cross the blood brain barrier It is thought that the cytotoxic action of 6-OHDA is associated with the ease of auto-oxidation, which is a complex process with the formation of a number of extremely reactive, potentially cytotoxic compounds such as quinones, hydrogen peroxide, superoxide- hydroxy- and other radicals Nerve terminals appear to be the most sensitive part of the neuron (Jonsson, 1980) Noradrenergic or dopaminergic neurons can be preferentially lesioned by the prior administration of selective uptake blockers In high concentrations 6-OHDA has been reported to lose its selectivity and damage serotonergic neurons as well (Breese and Cooper, 1977). DSP4 has been reported to be a neurotoxin producing longlasting selective degeneration of noradrenergic neurons arising in

the LC. DSP4 crosses the blood brain barrier and therefore can be administered intraperitoneally (Jonsson, 1980). DSP4 is accumulated intraneuronally through NA uptake sites and as with 6-OHDA affects nerve terminals primarily, however, degeneration is at a slower rate (Jonsson et al., 1981). It has been proposed that the toxic effect of DSP4 is via an alkylation reaction (Jonsson, 1980). The formation of a toxic metabolite by the action of MAO-B has also been suggested (Gibson, 1987).

1.3 NEUROTRANSMITTER RECEPTORS

1.3.1 Introduction

Neurotransmitter receptors are integrated within the structure of the cell membrane (Michell, 1987) and may be located postsynaptically in the somatodendritic region or on axons of neighbouring nerve cells (Kuhar, 1987). Activation of postsynaptic receptors leads to the initiation of action potentials (Section 1.1.1). Receptors on axon terminals located before the synaptic cleft have been denoted as presynaptic receptors (Göthert, 1985). If such receptors on a terminal nerve fibre are activated by the neurotransmitter released from the same nerve terminal, they are called autoreceptors (Göthert, 1985, Chiodo, 1988). These receptors have been distinguished from presynaptic heteroreceptors which are activated by transmitters released from neighbouring nerve terminals (Fig. 1.10). Autoreceptors have also been reported to be located in the somatodendritic region where activation of the autoreceptor inhibits firing of neurons and therefore decreases the release of transmitter (Göthert, 1985, le Douarin et al., 1986). Neurotransmitters influence cells by interacting with more than one type of receptor. Adrenergic receptors, sensitive to NA and adrenaline, have been subdivided into α_1, α_2, β_1 and β_2 subtypes (Göthert, 1985, Nahorski et al., 1985; Lorton and Davis, 1987; Boyajian et al., 1987; Rainbow and Bigeon, 1983). 5-HT receptors have also been subclassified according to their pharmacological characteristics and 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2, and 5-HT_3 subtypes have been described (Peroutka and Snyder, 1983; Invernizzi et al., , 1988, Neijt et al., 1986; Bischoff et al., 1988; Herrick-Davis et al., 1988). Two major subtypes have been

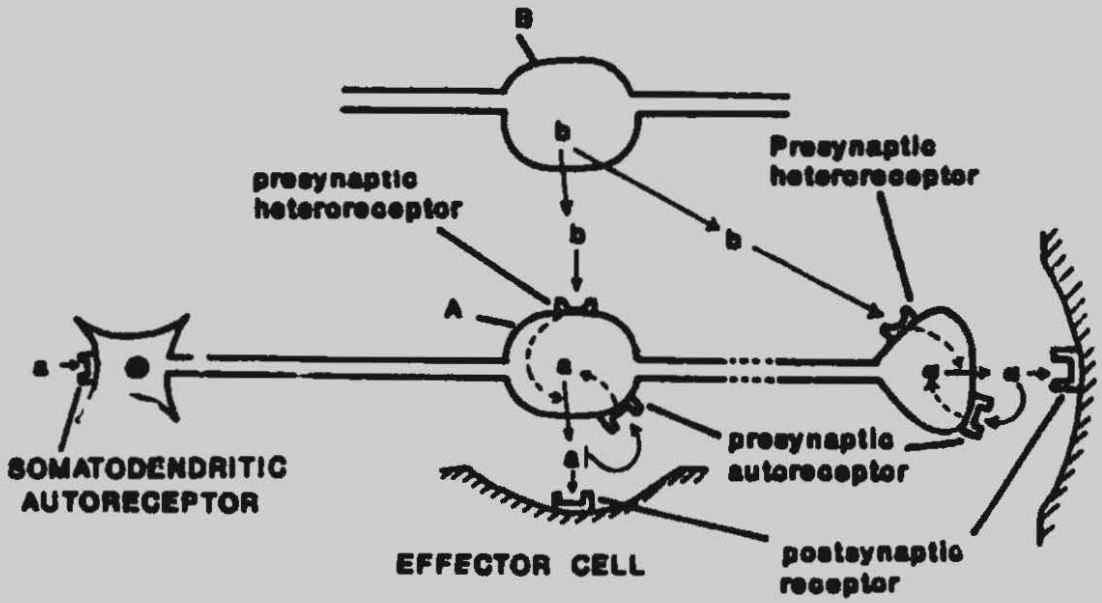

Fig. 1.10 Schematic representation of presynaptic auto- and heteroreceptors and somatodendritic autoreceptors localized on neuron "A" which releases neurotransmitter "a". The effector cell has a postsynaptic receptor for transmitter "a". Neuron "B" releases neurotransmitter "b". Presynaptic auto and heteroreceptors influence the release of "a".

described for DA receptors, the DA D1 and DA D2 receptors (Kebabian and Calne,1979, Stoof and Kebabian, 1981; Offermeier and van Rooyen, 1982). A variety of subtypes of DA receptors have previously been described. Of the DA D1, D2, D3, and D4 receptors proposed by Martres et al., (1982), the D3 and D4 subtypes have more recently been suggested to be the high affinity states of the D1 and D2 receptors defined by Kebabian and Calne, (1979) (Seeman and Niznik, 1988; Seeman et al., 1985). DA inhibitory (DAi) and excitatory (DAe) receptors have also previously been proposed (Cools and van Rossum, 1982). However, the present consensus among investigators is that the classification of DA receptors into DA D1 and D2 subtypes, as was originally proposed by Kebabian and Calne, (1972) is the most acceptable.

1.3.2 Dopamine D1 and D2 receptors

1.3.2.1 Properties

The characterization of DA D1 and D2 receptors has been greatly aided by the introduction of selective agonists and antagonists for these receptors. The most widely used selective DA D1 agonist and antagonist are SKF38393 and SCH23390 respectively Selective DA D2 receptor agonists include quinpirole (LY171555), LY141865, RU24213 and N-0437, while selective antagonists for the DA D2 receptor include sulpiride and Ro22-2586, (Clark and White, 1987; Niznik, 1987) The butyrophenones, spiperone, domperidone and haloperidol have a higher affinity for the DA D2 than the DA D1 receptor (Billard et al., 1984; Hyttel and Arnt, 1987) but are not as selective as sulpiride and Ro22-2586. Agonists with mixed DA D1 and D2 receptor affinity include apomorphine and amphetamine while antagonists with mixed DA D1 and D2 affinity include flupenthixol, pifluthixol and butaclamol (Table 1.1).

Separation of DA D1 and D2 receptors has been achieved by steric exclusion high performance liquid chromatography (HPLC) after solubilization of the receptor membrane (Dumbrille-Ross et al., 1985) and affinity chromatography has been used to isolate the DA D1 receptor (Gingrich

TABLE 1.1

Inhibition of [^{3}H]SCH23390 and [^{3}H]SPIPERONE binding by various agents

Compound	[^{3}H]SCH23390 K_i (nM)	[^{3}H]SPIPERONE K_i (nM)
Dopamine Receptor Antagonists		
SKF38393	18	9300
SCH23390	0 3	760
(cis) flupenthixol	4.3	0.8
(trans) flupenthixol	907	94
(cis) pifluthixol	2.9	1.0
(trans) pifluthixol	95	73
(+) butaclamol	14 6	1.8
chlorpromazine	74	8.2
fluphenazine	11 2	1.2
perphenazine	29.9	1.3
thioridizine	59	9.1
spiperone	8400	0 12
haloperidol	835	1.8
sulpiride (R)	30000	1102
sulpiride (S)	>100000	10
domperidone	10520	0.72
Dopamine Receptor Agonists		
dopamine	1450	2627
apomorphine	432	540
quinpirole	>5000	720
pergolide	100	0.65
Miscellaneous		
methysergide	217	116
ketanserin	1005	397
serotonin	44000	1655
noradrenaline	12000	21351

Adapted from Billard et al., (1984) and Andersen et al., (1985). K_i values were calculated using the equation: $K_i = IC_{50}/(1 + l/K_D)$ where L is the concentration of labelled ligand and K_D is the equilibrium dissociation constant.

et al., 1988; Niznik, 1987), providing a means by which the two DA receptor subtypes may be further elucidated The apparent in situ molecular size of DA receptors has been estimated using irradiation activation and photoaffinity labelling techniques (Gredal and Nielsen, 1987; Andersen and Nielsen, 1987; Amlaiky et al., 1987). From these studies DA D1 receptors

appear to be 72000-79000 daltons while DA D2 receptors appear to be between 93000 and 136000 daltons.

Both DA D1 and D2 receptors have been shown to exist in interconvertable high and low agonist affinity forms (Bzowej et al., 1985, Hamblin et al., 1984, Usdin, 1980). High agonist affinity receptor conformations have been reported to be induced by divalent cations such as Ca^{++} and Mg^{++} (Hamblin et al., 1984) whereas Na^{+} ions and guanyl nucleotides have been reported to lower the affinity of DA D1 and D2 receptors for agonists (Hess and Creese, 1986; Seeman et al., 1987, Creese et al., 1978) However, affinity shifts induced by cations do not appear to be related to the agonist or antagonist nature of the DA receptors (Urwyler, 1987).

DA D1 receptors have been shown to be situated postsynaptically while DA D2 receptors are located both pre- and postsynaptically (Filloux et al., 1987; Carlson et al., 1986; Vulto and Fowler, 1986, Calebresi et al., 1987). DA autoreceptors have been reported to be more sensitive to agonists than postsynaptic receptors (Chiodo, 1988, Timmermans and Thoolen, 1987).

Differences between DA D1 and D2 receptors have also been observed in functional studies Behavioural, electrophysiological and pharmacological studies have indicated that DA D1 and D2 receptors do not act independently of each other. In some cases the two receptors appear to have opposing effects while in others the effects appear to be synergistic

1.3.2.2 Functional studies

The functional activity of DA D2 receptors has been demonstrated by various behavioural studies. Injection of the agonists apomorphine and amphetamine cause increased locomotor activity in normal rats (Molloy et al., 1986), and rotation in 6-OHDA lesioned rats (Ungerstedt, 1971b). Chronic administration of DA D2 antagonists has been reported to result in catalepsy (Clark and White, 1987). Until recently the function of the DA D1 receptor was unclear (Laduron, 1981). However chronic administration of SCH23390 has been reported to result in

catalepsy (Morelli and di Chiara, 1985). Similarly, like the DA D2 receptor antagonist neuroleptics, the DA D1 receptor antagonist, SCH23390, has been reported to have antistereotypic effects (Christensen et al., 1984) and this has provided evidence for a function for the DA D1 receptor.

In behavioural studies the hypomotility produced by low doses of apomorphine has been attributed to DA autoreceptors (Radhakishun and van Ree, 1987). This effect can be blocked by DA D2 receptor antagonists but not DA D1 receptor antagonists indicating that the receptors are of the D2 subtype (Clark and White, 1987). The increased locomotor activity observed after higher doses of apomorphine has been reported to be due to postsynaptic dopaminergic activation (Clark and White, 1987).

Opposing effects of DA D1 and D2 receptor agonists on certain behaviours in rats have also been described. For example SKF38393 caused a dose dependent increase in oral movements such as chewing and tongue protrusions in the rat whereas quinpirole decreased oral activities (Johansson et al., 1987).

Behavioural studies provided early evidence that DA receptor subtypes may work in a synergistic manner. In rats with unilateral 6-OHDA lesions of the substantia nigra, the DA D1 receptor agonist, SKF38393, at a dose that did not itself cause turning, increased the contralateral rotation observed following administration of the DA D2 agonist, quinpirole (Robertson and Robertson, 1986; Arnt and Perregraard, 1987). It has also been reported that SKF38393, while almost inert alone, enhanced the expression of oral stereotypy (licking and biting) after combination with quinpirole (Arnt et al., 1987). Evidence from behavioural studies has led to the suggestion that DA D1 receptors play an enabling role in the expression of DA D2 effects (Arnt and Hyttel, 1988; Clark and White, 1987). However, prior activation of DA D2 receptors has also been suggested to be necessary for the expression of certain behaviours in rats. Morelli et al., (1987b) reported that contralateral turning after SKF38393 administration to 6-OHDA lesioned rats was only observed when the rats had previously been

exposed to apomorphine or quinpirole.

In normal rats DA D1 receptor agonists induce behaviours (increased oral movements) which are not dependent on DA D2 receptor stimulation. DA D2 receptor agonists also induce behaviours (increased locomotion, and low intensity stereotypic sniffing and rearing) independent of DA D1 receptor stimulation (Molloy et al., 1986; Arnt et al., 1988). It has therefore been proposed that the expression of certain behaviours may depend on a balance between DA D1 and D2 receptor stimulation (Clark and White, 1987). However, in these behavioural studies endogenous DA can also play an enabling role.

Electrophysiological studies have also provided evidence that DA autoreceptors are of the DA D2 subtype. It has been demonstrated that DA receptors located on the somatodendritic area of midbrain DA neurons control the firing rate of neurons (Chiodo, 1988; White, 1986). These receptors have been reported to be of the DA D2 receptor subtype since application of DA agonist apomorphine or quinpirole caused a decrease in neuron firing (White, 1986; Trulson et al., 1987; Timmermans and Thoolen, 1987). In another study LY141865, administered intravenously was reported to cause a biphasic effect on the firing rate of nucleus accumbens neurons. An initial increase in firing rate was observed and was proposed to be due to autoreceptor disinhibition occurring at the more sensitive autoreceptors (Hu and Wang, 1988). However, postsynaptic DA D1 receptors have also been suggested to be able to modulate the activity of DA neurons through long-loop feedback mechanisms (Carlson et al., 1987b).

Electrophysiological studies have also highlighted some differential effects of DA D1 and D2 receptors. Bath applied SKF38393 or DA but not quinpirole inhibited action potentials of striatal neurons produced by electrical stimulation (Calebresi et al., 1987). In the nucleus accumbens iontophoretically applied SKF38393 reduced the activity of a subpopulation (38%) of neurons, measured by an extracellular recording technique. SCH23390 but not sulpiride was shown to block this effect. The iontophoretic application of LY141865 or quinpirole inhibited the activity of 75% of the neurons tested and this effect was blocked by sulpiride and

not by SCH23390 (White and Wang, 1986) Iontophoretic application of DA has been reported to produce both inhibition and an increase of spontaneous firing in the nucleus accumbens. The inhibition was mimicked by the DA D1 agonist SKF3839 and the DA D2 agonist bromocriptine was observed to result in excitation These inhibitory DA D1 and excitatory DA D2 receptors have been reported to exist on the same neuron in the caudate nucleus of the cat (Ohno et al., 1987). Intracellular recording methods have provided evidence that DA D1 receptor activation results in hyperpolarization of the neurons with an increase in potassium conductance, whereas DA D2 receptor activation caused depolarization with the associated decrease in potassium conductance (Uchimura et al., 1987). These results support the behavioural studies indicating opposing effects mediated via DA D1 and D2 receptors

However, there is also electrophysiological evidence indicating that both DA D1 and D2 receptor activation is necessary for full expression of postsynaptic effects (Carlson et al., 1987b, Weick and Walters, et al., 1987) and on the basis of these results as well as on behavioural evidence the proposal of an enabling function of the DA D1 receptor has been supported (White, 1987 Clark and White, 1987). Kelly and Nahorski, (1987) have suggested that endogenous DA activating DA D1 receptors may similarly have an enabling role

In confirmation of the results of behavioural and electrophysiological studies, autoreceptors modulating DA synthesis and metabolism have also been shown to be of the DA D2 subtype (Magnusson et al., 1987, Wolf et al., 1986, Kilts et al., 1987, Vulto and Fowler, 1986; Galloway et al., 1986) and not the DA D1 subtype (Watanabe et al., 1987; Imperato et al., 1988; Zetterström et al., 1986). However, basal DA synthesis has been reported to be decreased by the selective DA D1 receptor agonist, SKF38393, but this does not appear to be a receptor mediated effect since the effect is not blocked by the DA D1 or D2 receptor antagonists SCH23390 and sulpiride (Clark and White, 1987).

DA release modulating autoreceptors have also been reported to exhibit the characteristics of the DA D2 receptor subtype (Herdon et al., 1986). DA receptors modulating acetylcholine

(ACh) release are present in the striatum and nucleus accumbens. These inhibitory receptors are located either postsynaptically on cell bodies (Herrling et al., 1980) or presynaptically on the nerve terminals of cholinergic interneurons (Gold and Bluth, 1985). The latter have been reported to be of the DA D2 receptor subtype (Dawson et al., 1986). The DA receptor mediated inhibition of ACh release is generally accepted as an in vitro model of the presynaptic DA heteroreceptor function (Timmermans and Thoolen, 1987; Gold and Bluth, 1985). Several studies have shown opposing effects of DA D1 and D2 receptors in this model. The systemic administration of the DA D2 receptor agonist LY141865 was reported to increase the K^+-evoked release of [^{3}H]ACh while the DA D1 receptor agonist SKF38393 was without effect (Scatton, 1982). In the same study DA D2 antagonists induced a decrease in the K^+-evoked release of [^{3}H]ACh whereas SCH23390 had no significant effect. Fage and Scatton (1986) reported an increase in rat striatal ACh levels after SCH23390 administration while DA D2 or mixed antagonists caused a decrease. SCH23390 was reported to block the ability of sulpiride to decrease ACh release and to enhance the increase induced by LY141865. In an in vitro superfusion study Saller and Salama, (1986) showed potentiation of K^+-stimulated release of [^{3}H]ACh by the DA D2 receptor antagonist haloperidol which was antagonized by the DA D1 receptor antagonist SCH23390 although SCH23390 was by itself without effect.

Stimulation of DA D1 and D2 receptors has also been reported to produce opposing effects on K^+-stimulated or spontaneous release of [^{3}H]gamma-aminobutyric acid (GABA) (Girault et al., 1986; Starr, 1987). The stimulation of DA D1 receptors caused increases in [^{3}H]GABA release while DA D2 receptor stimulation inhibited its release.

From the many behavioural, electrophysiological and biochemical studies performed there has been sufficient evidence for DA D1 as well as D2 receptor function in the brain. The evidence obtained also suggests interactions between DA D1 and D2 receptors in both opposing and synergistic ways and that DA D2 receptors require DA D1 receptor stimulation for the expression of certain functional activities.

1.3.2.3 Second messengers

There are several methods by which signals arising from the activation of neurotransmitter receptors in the membrane may be transmitted to the cell interior to control physiological responses Some receptor proteins themselves transmit the signal. Others send their messages to the cell interior by controlling enzymes associated with the membrane Another mechanism controls the formation of intracellular second messenger molecules. This process depends on the transformation of a number of proteins in the membrane from where the information is transmitted to small molecules or ions called second messengers, in the cytoplasm of the cell (Michell, 1987). Compared to the number of receptors and neurotransmitters, there are very few second messengers which are able to regulate a vast variety of physiological and biochemical processes. Some of the second messenger systems include cyclic adenosine-3,5-monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), phosphinositides and the Ca^{++}/calmodulin complex (Klee et al., 1980). Many receptors, including DA receptors, control cells by influencing adenylate cyclase activity to catalyse either the stimulation or the inhibition of cAMP formation This takes place via stimulatory or inhibitory receptors which communicate with adenylate cyclase through Gi and Gs proteins in the membrane which are activated when bound to guanosine triphosphate (Fig 1 11).

DA D1 receptors stimulate adenylate cyclase by interacting with the stimulatory G protein (Olianas and Onali, 1987) and DA D2 receptors are coupled to the inhibitory G protein (Kelly et al., 1987; Niznik, 1987). cAMP activates a specific protein kinase which phosphorylates various proteins (Michell, 1987). DA stimulated cAMP dependent protein kinase has been reported to phosphorylate the protein DARPP-32, which is present in neurons containing DA D1 receptors (Hemmings et al., 1987). DA D2 receptor stimulation has also been reported to lead to an decrease in inositol phosphates (Pizzi et al., 1987) and an inhibition of Ca^{++} influx (Memo et al., 1987), providing evidence for more than one mechanism of trandsduction across cell membranes following DA D2 receptor activation.

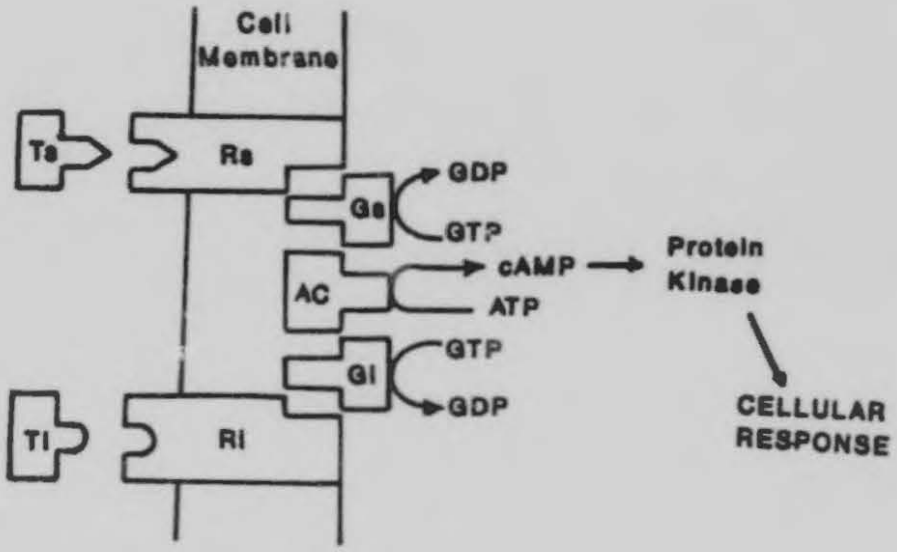

Fig. 1.11 Schematic model of neurotransmitter-sensitive adenylate cyclase system.

AC, adenylate cyclase; cAMP, adenosine 3,5-cyclic phosphate; ATP, adenosine triphosphate; GTP, guanosine triphosphate; T, transmitter; I, inhibitory; S, stimulatory; R, receptor; G, regulatory protein.

DA D2 receptors have also been shown to be independent of adenylate cyclase (Kebabian and Calne, 1979; Stoof and Kebabian,1981). For example, Stoof and Verheijden, (1986) have suggested that DA D2 receptors in the nucleus accumbens are not linked to adenylate cyclase as formation of cAMP following DA D1 receptor stimulation was not inhibited by selective DA D2 receptor agonists in that tissue, whereas inhibition did occur in the striatum.

The presence of "spare" DA D1 receptors has been suggested by several authors. In one study the percentage of DA D1 receptor mediated adenylate cyclase activity was shown to be greater than the DA D1 receptor density after recovery from irreversible block using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (Battaglia et al., 1986). In another study determination of DA D1 receptor number and DA sensitive adenylate cyclase in various cellular fractions of rat striatum after centrifugation resulted in a greater number of DA D1 binding sites being measured (Mailman et al., 1986). It has been suggested that some of these DA D1 receptors are non functional (Laduron, 1981) and that not all of the DA D1 receptors are linked to cAMP production (Mailman et al., 1986). It has also been reported that only 60% DA D1 receptor occupation is necessary for full agonist stimulation of adenyate cyclase and therefore the presence of spare receptors may enhance neurotransmission by reducing the effective concentration of DA required (Hess and Creese, 1987). DA D1 receptor synthesis and turnover has been reported to be greater than that of DA D2 receptors, particularly in the nucleus accumbens (Fuxe et al., 1987; Fukuchi et al., 1986) indicating that changes in demand for DA D1 activity and function can be more rapidly modulated.

The different effects activation of DA D1 and D2 receptors has on adenylate cyclase activity and subsequent cAMP formation demonstrates a basic mechanism which may be responsible for the opposing effects of DA D1 and D2 receptors in behavioural and electrophysiological studies.

1.3.2.4 Localization

DA D1 and D2 receptor localization in the brain has been studied using autoradiographic techniques both in vitro and in vivo (Loopuijt et al., 1987, Closse et al., 1988; Dubois et al., 1986; Savasta et al., 1987a; Bouthenet et al., 1987). Investigation of the distribution of DA D1 and D2 receptors in human and animal brains has also been made possible by the use of positron emission tomography (Arnett et al., 1986; Gjedde and Wong, 1987). The distribution of DA D1 receptors has been shown to overlap with that of DA D2 receptors in a number of DA-rich brain areas but there are several areas where high densities of DA D1 receptors were reported but where undetectable levels of DA D2 receptors were observed.

DA D1 receptors have been reported to be located in high concentrations in the substantia nigra, caudate putamen, nucleus accumbens and olfactory tubercle (Aiso et al., 1987; Savasta et al., 1986; Dubois et al., 1986; de Keyser et al., 1988). Aiso et al., (1987), using [^{125}I]SCH23982, reported levels of DA D1 receptors in the substantia nigra pars reticulata to be three times higher than those in the caudate putamen but in contrast Dubois et al., (1986), using [^{3}H]SKF38393, reported similar levels in these areas and Savasta et al., (1986) reported higher levels in the caudate putamen than in the substantia nigra where the density of DA D1 receptors was observed to be highest in the pars compacta (Table 1.2). In the substantia nigra DA D1 receptors have been reported to be highest in the medial and lowest in the lateral area (Altar and Marien, 1987; Aiso et al., 1987). The density of DA D1 receptors has been reported to be higher in the caudate putamen than in the nucleus accumbens (Savasta et al., 1986).

High densities were also apparent in the nucleus interstitialis striae terminalis, the anterior olfactory nucleus, the entopeduncular nucleus, the subthalamic nucleus, the claustrum and the amygdalohippocampal area. Intermediate levels of DA D1 receptors were observed to be located in the anteromedial and suprarhinal areas of the frontal cortex, particularly layers V and VI. This is in agreement with other authors (Dawson et al., 1987). The basolateral, medial and lateral amygdaloid nuclei, the endopiriform nucleus, the primary olfactory cortex, the

TABLE 1.2

Quantitative autoradiographic analysis demonstrating the distribution of DA D1 and D2 receptor binding in the rat brain

Area	[^{3}H]SKF38393 (fmol/mg protein)	[^{3}H]SCH23390 (fmol/mg protein)	[^{3}H]NPA (fmol/mg protein)
Frontoparietal cortex	223± 20	NR	ND
Nucl. olfactory anterior	464± 45	1543± 77	ND
Nucleus accumbens	727± 70	2458± 122	255± 25
Olfactory tubercle	934± 91	2989± 149	702± 55
Septum lateralis	440± 40	NR	143± 14
Primary olfactory cortex	722± 70	408± 20	ND
Caudate putamen	1312± 120	3079± 153	1516± 104
Globus pallidus	238± 18	345± 17	42± 6
Corpus callosum	11± 2	NR	13± 2
Nucl. amygd. centralis	631± 62	NR	NR
Thalamus, ventroposterior	402± 40	227± 11	ND
Endopiriform nucleus	679± 65	160± 8	14± 2
Habenula	436± 42	NR	329± 30
Hypothalamus, dorsomedial	421± 42	NR	37± 4
Arcuate nucleus	151± 15	NR	ND
Median eminence	48± 4	NR	17± 2
Hippocampus, dentate gyrus			
molecular layer	343± 30	190± 10	88± 5
granular layer	276± 17	230± 12	ND
Subthalamic nucleus	393± 35	1040± 50	95± 7
Substantia nigra			
pars reticulata	891± 81	2710± 136	236± 20
pars compacta	1028± 101	1967± 98	182± 16
Ventral tegmental area	187± 30	146± 7	125± 7
Superior colliculus	467± 46	340± 17	104± 8
Cerebellum, granular	410± 51	NR	ND
molecular	657± 65	NR	ND

Adapted from Savasta et al., (1986) and Camus et al., (1986)
ND = not detected, NR = not recorded

globus pallidus, the superficial layer of the superior colliculus, the nucleus amygdaloideus corticalis and the dorsal hippocampus were also reported to contain high levels of DA D1 receptors (Savasta et al., 1986) Moderate levels were reported to be present in the thalamus, hypothalamus, habenula, VTA, posterior cingulate and entorhinal cortices, the supragenual DA terminal system and the molecular layer of the cerebellum However, DA D1 receptors have also been reported to be absent in the VTA (Altar and Marien, 1987, Altar and Hauser, 1987)

Areas where high densities of DA D1 receptors were reported to be located but where undetectable levels of DA D2 receptors were found include the frontoparietal and primary olfactory cortices, the nucleus olfactorius anterior, nucleus amygdaloideus centralis, the globus pallidus, the ventroposterior thalamus, the endopyriform nucleus, the arcuate nucleus and the cerebellum (Dubois et al., 1986). Dawson et al., (1986) have reported that DA receptors in the cerebral cortex are postsynaptic DA D1 receptors, and evidence for the lack of synthesis modulating DA D2 autoreceptors is also provided by Kilts et al., (1987) and Galloway et al., (1986). The distribution of DA D1 receptors therefore appears to be more widespread in the brain than that of DA D2 receptors

The striatal subregions have been studied in more detail and the density of DA D1 receptors was found to be greater in the ventrolateral sector and medial margin of the striatum than in the ventromedial and dorsolateral areas (Savasta et al., 1986, Savasta et al., 1987a). Rostrocaudally, a decrease (Savasta et al., 1986) or a bell shaped distribution of DA D1 receptors has been reported in the striatum (Savasta et al., 1987a). DA D1 receptors have also been reported to be present on glial cells (Dubois et al., 1986) It therefore appears that, apart from the few areas indicated, the density of DA D1 receptors generally follows the distribution of DA terminals in the brain.

6-OHDA lesions of the substantia nigra or the mesostriatal dopaminergic projection have been reported to have no effect on the DA D1 receptor density in the striatum (Savasta et al., 1987a, Filloux et al., 1987b; Altar and Marien, 1987) or the nucleus accumbens (Savasta et al., 1987a), and these findings have therefore provided evidence that DA D1 receptors are not located on dopaminergic terminals. It has been suggested that they may be located postsynaptically on intrinsic neurons in the striatum (Savasta et al., 1987a). Unlike DA D2 receptors which have been reported to increase by more than 30% thirty days after nigrostriatal lesions, these postsynaptic DA D1 receptors do not appear to increase in number following similar lesions (Savasta et al., 1987a) However, in contrast to these findings, one study has provided evidence

of an increase in both adenylate cylase activity and DA D1 receptor density in the striatum fourteen days after 6-OHDA lesion of the MFB (Porceddu et al., 1987).

In the substantia nigra DA D1 receptors have been reported to reside on terminals of striatonigral neurons, particularly in the medial and central parts of the pars reticulata (Altar and Marien, 1987) as quinolinic acid and ibotenic acid lesions of the striatum caused significant loss of DA D1 receptors in these areas (Altar and Marien, 1987; Filloux et al., 1987b). 6-OHDA lesions of the substantia nigra resulted in a decrease in DA D1 receptors in the pars compacta where the DA D1 receptors have been suggested to be located on DA cell bodies (Savasta et al., 1986), whereas in the pars reticulata DA D1 receptors may be situated on terminals of non-dopaminergic neurons such as GABA and substance P neurons (Porceddu et al., 1986; Savasta et al., 1987a)

The highest levels of DA D2 receptors in the brain have been reported to be present in the caudate putamen, nucleus accumbens and globus pallidus (Camus et al., 1986, van der Weide et al., 1987b, Gehlert et al., 1984; Richfield et al., 1986), (Table I 2).

High levels of DA D2 receptors were also reported to be present in the olfactory tubercle and associated islands of Calleja, the subthalamic nucleus, the bed nucleus of the stria terminalis, the substantia nigra pars compacta and the VTA (Bouthenet et al., 1987; Closse et al., 1988) Intermediate to low levels were observed in the hippocampus, insular and cingular cortices and in the occipito-temporal gyrus, while almost undetectable levels were found in the anterior frontal cortex. DA D2 receptor densities have been reported to be similar in the caudate nucleus and the nucleus accumbens (Camus et al., 1986, Bouthenet et al., 1987). However, in one study, the DA D2 receptor density in the caudate putamen using [^{3}H]NPA was found to be six times higher than in the nucleus accumbens (Savasta et al., 1986)

Within the caudate nucleus the DA D2 receptor density was higher laterally than medially (Camus et al., 1986, Joyce and Marshall, 1987), however, high densities of DA D2 receptors

have also been reported to be found at the medial border of the caudate putamen (Bouthenet et al., 1987). Along the rostrocaudal axis the greatest concentration of DA D2 receptors was present in the midbody of the nucleus These heterogeneities could account for the differences observed between different studies when only one value is reported

The density of DA D2 receptors generally paralleled that of DA innervation, however there appear to be areas in which DA D2 receptors are found but where DA innervation has not been established such as layers I-III and V-VI of the cerebral cortex, the cerebellum and the molecular layer of the hippocampus. In addition there are areas in which DA D2 receptors could not be detected in spite of a well established DA innervation, for example in areas of origin or termination of the tuberohypophyseal and incertohypothalamic dopamine systems and the amygdaloid complex (Bouthenet et al., 1987).

In the caudate putamen DA D2 receptors have been reported to reside on the terminals of DA neurons arising in the substantia nigra (Morelli et al., 1987; Palacios, 1986). They have also been reported to be located on intrinsic striatal neurons (Joyce and Marshall, 1987; Palacios, 1986, Filloux et al 1987a; Van der Weide et al., 1987a) DA D2 receptor density was found to correspond to acetylcholinesterase positive neurons and these receptors have been reported to be located on striatal cholinergic neurons (Joyce and Marshall, 1987; Gold and Bluth, 1985, Dawson et al., 1988) DA D2 receptors have also been reported to be located on glutaminergic projections from the cortex (Dawson et al., 1988, Palacios, 1986) However Trugman et al., (1986) have provided evidence following kainic acid lesion of the striatum that DA D2 receptors do not exist on terminals of the corticostriatal pathway. In the substantia nigra pars compacta and VTA DA D2 receptors have been reported to be located on perikarya and dendrites of DA neurons as 6-OH DA lesions of the MFB reduced DA D2 receptors by about 50% in these areas (Bouthenet et al., 1987; Murrin et al., 1979; Filloux et al., 1987a)

A heterogeneous distribution of DA D1 and D2 receptors in the brain has therefore been demonstrated There appears to be some overlap in the regional localization of the two receptor

subtypes and in general the localization of DA receptors parallels the areas of highest DA innervation, however, certain exceptions have been noted. DA D1 receptor distribution appears to be more widespread than the DA D2 receptors in the brain and the absolute DA D1 receptor levels are also higher than those of DA D2 receptors (Table 1.2). Hyttel and Arnt, (1986) reported that the DA D1 receptor density was 2-6 times higher than the DA D2 receptor density in the rat striatum and that the ratio increased via the striatum to the olfactory tubercle. The distribution of DA receptors has been studied in detail in areas such as the caudate nucleus and the substantia nigra, however, little has been reported on the exact localization of DA D1 and D2 receptors in other areas such as the nucleus accumbens.

CHAPTER 2

DEPRESSION

2.1 INTRODUCTION

Depressive illness represents a major health problem with estimates of 13 to 20% of the population suffering from depressive symptoms at any given time (Gold et al., 1988). Primary affective disorders, those occurring in people who have no other psychiatric diagnosis, have been described as bipolar or unipolar (Schlesser et al., 1980). When primary depressive illness is associated with mania it is defined as bipolar and generally occurs in the early 20's. In the absence of mania the illness is termed unipolar and most often starts in middle life These major affective disorders have been reported to be heritable (Gold et al., 1988a; Schlesser et al., 1980), recurrent, syndromal illnesses with both psychological and biologic components Untreated episodes usually last from 7 to 14 months but may continue for 2 years or more. Symptoms include depressed mood, withdrawal of interest, feelings of worthlessness, anorexia or hyperphagia, insomnia or hypersomnia and altered timing of the cycle of rest and activity (Gold et al., 1988). From the evidence of recent studies it also appears that major depression consists of subgroups such as those including the syndrome, melancholia This disorder is characterized by a consistent association with symptoms of hyposomnia, anorexia, decreased sexual interest, endocrine abnormalities and diurnal variation in mood and can occur in patients with both bipolar or unipolar depression (Gold et al., 1988a). Different subgroups of major depression have also been suggested by the demonstration of different biochemical abnormalities (Peabody et al., 1987).

Several diagnostic methods have been employed in the identification of depression. These include the Diagnostic and Statistical Manual of Mental Disorders, III (1980) and the Research Diagnostic Criteria (Spitzer et al., 1978) which are used to establish unipolar/bipolar and

primary/secondary criteria. The degree of depression is assessed by the Hamilton Depression Rating Scale (Hamilton 1960) and the Beck self-evaluating scale (Beck et al., 1961).

In major affective disorders symptoms have also been reported to reflect alterations in the hypothalamic centres, for example food intake, libido and circadian rhythms (Gold et al., 1988b). There appears to be increased activity of the hypothalamic-pituitary-adrenal axis in depressive illnesses (Schlesser et al., 1980; Gold, et al., 1988b; de Villiers et al., 1987). The most specific measure of this is the dexamethasone suppression test in which the decrease in serum cortisol levels is determined on the day after administration of 1mg dexamethasone. Different subgroups of major depression have also been demonstrated by this test. For example, it has been reported that some patients with primary unipolar depression did not show suppressed serum cortisol levels after dexamethasone administration (Schlesser et al., 1980).

2.2 BIOCHEMICAL ABNORMALITIES

Several biochemical abnormalities have been reported in depressive disorders. Plasma NA levels have been reported to be increased in patients with major affective disorder being similar in manic, bipolar or unipolar disorders. This sympathetic hyperactivity was suggested to contribute to some of the somatic complaints of depressed patients which include dizziness, anxiety, constipation, palpitations and hyperactivity (Lake et al., 1982). However, Roy et al., (1985b) reported that plasma NA was only increased in patients with major depressive episode with melancholia, when compared to controls. Depressed patients without melancholia had NA levels comparable with controls. Bipolar patients with melancholia had lower NA levels than unipolar patients with a similar history of melancholia. From these results it would appear that the increase in NA levels was confined to a subgroup of patients, those with unipolar depression with melancholia. It was also reported that among unipolar patients with melancholia, nonsuppressors in the dexamethasone test had higher NA levels than

suppressors (Roy et al., 1985b). A biological significance of melancholia was therefore demonstrated The higher NA levels in melancholics were also related to self-rated anxiety, supporting the suggestion of correlation with somatic complaints since plasma NA reflects the sympathetic nervous system activity.

MHPG is the major metabolite of brain NA and it has been widely accepted that changes in brain noradrenergic activity will be reflected in corresponding changes in MHPG formation (Maas, 1984) MHPG levels have therefore been measured in the urine, plasma and cerebrospinal fluid (CSF) in a number of studies. MHPG levels have been reported to be significantly lower in the urine of patients with bipolar manic-depressive or schizoaffective depressions than in controls or patients with unipolar depression Those with high MHPG also had high free cortisol in the urine (Schatzberg et al., 1982). Consistent with the previous study, urinary MHPG was also reported to be lower in bipolar depressed patients than in those with unipolar depression (Schildkraut et al., 1978) Increased plasma NA and MHPG levels were also reported in patients with unipolar depressive disorder by de Villiers et al., (1987) These results support the suggestion of higher noradrenergic activity in patients with unipolar depression

Plasma MHPG levels have been measured after the dexamethasone suppression test and were found to be increased in those patients showing nonsuppression of cortisol (Jimerson et al., 1983) This showed a correlation between dexamethasone resistance and adrenergic activity. These findings were confirmed by Roy et al., (1988) who reported a correlation between urinary and CSF levels of MHPG and plasma cortisol levels after dexamethasone administration. CSF MHPG levels were higher in nonsuppressors after dexamethasone also suggesting that dysregulation of the noradrenergic system and the hypothalamic-pituitary-adrenal axis occur together in this subgroup of depressed patients Rubin et al., (1985) also reported that plasma MHPG levels were higher in nonsuppressors after the dexamethasone test.

A consistent biochemical abnormality which has been reported to occur in patients with major

depression is increased plasma cortisol le de Villiers et al., 1987, Gold et al., 1988b, Schlesser, 1980). These findings have supported evidence for increased activity of the hypothalamic-pituitary-adrenal axis and provided an explanation for the related symptoms.

The anticholinesterase, physostigmine, has been reported to cause increases in cortisol levels and it has been suggested that increased cortisol levels could be a result of increased cholinergic activity (Schatzberg et al., 1982). High levels of pseudocholinesterase have also been reported in anxiety-related psychiatric conditions (Aizenberg et al., 1989) which may indicate a relationship between the cholinergic and adrenergic systems.

CSF concentrations of HVA and 5-HIAA have been determined to provide a measure of DA and 5-HT metabolism in the central nervous system (van Praag, 1982) Lower levels of CSF 5-HIAA have been reported in depressed patients with melancholia than in controls. HVA levels were also found to be decreased in these patients Contrary to previous reports, no significant differences in MHPG were found (Asberg et al., 1984). Low 5-HIAA and HVA levels in patients with major depressive disorder were also reported by Peabody et al., (1987). A positive correlation was demonstrated between the 5-HIAA and HVA levels in these patients The correlation between CSF 5-HIAA and HVA levels has also been reported to occur in controls, however, the clinical significance of this association is not clear. Depression which is characterized by low 5-HIAA levels, has been linked to patients with suicidal behaviour (Peabody, et al., 1987, Asberg et al., 1984; van Praag, 1982). These results suggest a disturbance in central 5-HT metabolism in certain types of depression, and have been suggested to be a predisposing factor in depression (van Praag, 1982).

In patients with major depressive disorder with melancholia, CSF levels of DOPAC and HVA were found to be significantly lower than a combined group of patients with major depressive episode or dysthymic disorder. In agreement with previous studies the decreased HVA levels were significantly correlated with decreases in 5-HIAA levels (Roy et al., 1986). From statistical analyses, it has been proposed that 5-HIAA levels "control" HVA levels. 5-HT

turnover appears to have a regulatory action over DA turnover, although the functional meaning of this observation is not clear (Agren et al., 1986). However, these results provide evidence that diminished dopaminergic and serotonergic function may be involved in depressive illness. However the results of one study contrasts with these findings Increased CSF HVA levels were reported in depressed patients with melancholia when compared with neurological controls (Gjerris et al., 1987).

There appears to be sufficient evidence that biochemical abnormalities occur in major depressive disorders These findings have aided the diagnosis of the subtypes of depression and have led to the development of several theories of depression. The demonstration of certain biochemical abnormalities in depression has also resulted in the introduction of more specific drugs for its treatment

2.3 ANTIDEPRESSANT TREATMENT

One of the major effects of antidepressant treatment is the potentiation of monoaminergic synaptic function. MAO inhibitors achieve this by inhibiting the metabolism of monoamine neurotransmitters. Tricyclic antidepressant drugs block the neuronal uptake of these monoamines from the synaptic cleft (Green, 1988, Richelson and Pfenning, 1984) Therefore, in general, antidepressant drugs appear to share a common mechanism of action

The inhibition of MAO, which is present in the mitochondria of the nerve terminals, results in an elevation in the concentration of neurotransmitter available for release at the synapse. The initial effect does not appear to produce marked alteration in neurotransmitter levels in the synapse, and acute administration of the MAO inhibitor, clorgyline, has been reported to have little or no effect on neuronal events in the central nervous system (Finberg, 1987). However, chronic treatment with MAO inhibitors has been reported to result in increased plasma NA levels and there is evidence that catecholamine turnover is decreased after chronic treatment with either MAO inhibitors or tricyclic antidepressant drugs (Finberg, 1987, Karoum et al.,

1984). In support of these findings it has also been reported that after chronic desipramine and zimelidine treatment CSF and urine MHPG levels are reduced (Linnoila et al., 1986)

Tricyclic antidepressant drugs have been reported to exhibit different selectivities in the blockade of NA, 5-HT and DA uptake (Richelson and Pfenning, 1984). For example, desipramine and maprotyline have been observed to be more selective at blocking the uptake of NA than 5-HT or DA, while citalopram, zimelidine and fluoxetine more selectively blocked 5-HT uptake (Hyttel and Larsen, 1985, Richelson and Pfenning, 1984, Fuller and Wong, 1987) Amineptine (Bonnet et al., 1987) and bupropion have been shown to be relatively selective DA uptake blocking drugs (Richelson and Pfenning, 1984, Table 2.1) Plasma levels of NA have been reported to be increased after the acute administration of relatively selective NA uptake inhibitors such as desipramine, an effect that was suggested to be due to decreased clearance of the drug (Potter et al., 1987). When administered acutely, desipramine has also been reported to result in decreased brain levels of the NA metabolite, MHPG, indicating decreased turnover of NA (Bareggi et al., 1978). It has been suggested that a negative feed-back mechanism caused by the increased concentration of neurotransmitter in the synaptic cleft that occurs after monoaminergic uptake inhibition, decreases the synthesis and turnover of the transmitter by inhibition of neuronal firing (Finberg, 1987, Hyttel and Larsen, 1985) A close correlation appears to exist between uptake blockade of a particular transmitter and inhibition of its synthesis (Carlsson and Lindqvist, 1978) Several different neurotransmitter receptors have been shown to be directly antagonized by antidepressant drugs (Richelson, 1988, Richelson and Nelson, 1984). Tricyclic antidepressant drugs have been reported to show moderate affinity for histamine H_1 receptors, muscarinic receptors and α_1-adrenoceptors and weak affinity for α_2-adrenoceptors, DA D2 and 5-HT_2 receptors Some of the side effects of antidepressant drug treatment such as tachycardia, dizziness, tremor, sedation, dry mouth and blurred vision have been attributed to interactions with these receptors (Richelson, 1988) MAO inhibitors have been reported to have relatively weak affinity for all neurotransmitter receptors tested (Richelson, 1988)

TABLE 2.1

Inhibition constants (K_i) for blockade of monoamine uptake by antidepressant drugs and related compounds.

Compound	K_i (nM) for blockade of uptake of:		
	Noradrenaline (NA)	Serotonin (5-HT)	Dopamine (DA)
Desipramine	1	340	5200
Protryptyline	1	280	1850
Nortryptyline	4	260	1700
Chlorimipramine	28	5	1800
Imipramine	13	42	5110
Amitriptyline	24	66	2300
Doxepin	19	280	5700
Citalopram	4000	1	28000
Fluoxetine	280	12	1600
Zimelidine	3000	72	4400
Nomifensine	5	1280	51
Bupropion	2300	15600	630
GBR 12783	ND	ND	1.8*
Mianserin	42	2300	16200
Iprindole	640	330	3200

Adapted from Richelson and Pfenning, (1984) * IC_{50} value

Direct monoaminergic stimulation by the administration of the α_2-adrenoceptor agonist, clonidine, and the DA agonist, piribedil, has also been reported to result in antidepressant effects (Post, 1978).

Approximately 80% of patients respond to treatment with antidepressant drugs (Wägner and Klein, 1988; Kessler, 1978). One of the reasons for the failure of response in some patients has been reported to be interindividual variations in metabolism and a wide range of antidepressant drug doses has been used (Kessler, 1978). There is also evidence that some patients respond to one drug and not to another and it has been suggested that the biochemical status of the patient can be used in the selection of the antidepressant drug for therapy (Kessler, 1978; Asberg and Wägner, 1986). For example, it has been reported that patients who have low CSF MHPG levels respond better to drugs selectively blocking NA uptake while those with low 5-HIAA or HVA levels respond better to therapy with 5-HT selective blocking

antidepressant drugs (Asberg and Wägner, 1986)

The blockade of uptake by tricyclic antidepressant drugs and the reduction of monoamine catabolism by MAO inhibitors occur relatively rapidly whereas the clinical response of antidepressant drugs generally requires 2-3 weeks to become evident It has therefore been suggested that secondary adaptive changes in neurotransmitter receptors after chronic treatment with antidepressant drugs are necessary for the therapeutic effects to be evident and that the time needed for these changes to occur may account for the delayed onset of antidepressant efficacy (Fuller and Wong, 1987).

One of the most universal effects of antidepressant drugs, when administered repeatedly, is a decrease in the number of β-adrenergic receptors in the cerebral cortex This has been suggested not simply to result from adaptive changes following increased synaptic concentrations of NA since down-regulation of β-adrenoceptors occurs after electroconvulsive therapy, which does not result in decreased synthesis of NA acutely (Green, 1987) Down regulation of β adrenoceptors has also been suggested to be related to increased agonist levels (Finberg, 1987, Banerjee, 1977) However, intact serotonergic innervation of the cortex has been reported to be necessary for this effect to take place, indicating that 5-HT plays a permissive role in the compensatory changes in β-adrenoceptors (Green, 1987, Kotowski, 1981, Potter, 1987, Manji and Brown, 1988) Chronic antidepressant treatment has been reported to result in subsensitivity of α_2-adrenoceptors (Green, 1987, Kotowski, 1981) However, radioligand binding studies used to determine the α_2 adrenoceptor number have provided contradictory results (Green, 1987; Kotowski, 1981, Katona et al., 1987) This may be due to the presence of binding sites being located both pre- and postsynaptically Chronic administration of antidepressant drugs has also been reported to cause a decrease in the number of 5-HT_2 receptors in the frontal cortex (Green, 1987, Potter et al., 1987) and their function There is also behavioural evidence that 5-HT_{1A} receptors become subsensitive after chronic antidepressant treatment and 5-HT_{1A} agonists have been shown to possess

antidepressant properties (Kennet et al., 1987)

Behavioural studies have provided evidence that chronic antidepressant treatment leads to an increase in DA transmission (Spyraki et al., 1982, Willner, 1983), which has been suggested to be due to supersensitivity of postsynaptic DA receptor However, radioligand binding studies have provided evidence that chronic treatment with antidepressant drugs causes no change in the DA D2 receptor number or affinity in the striatum or forebrain of the rat (Rehavi et al., 1980, Peroutka and Snyder, 1980, Klimek and Nielsen, 1987) In contrast, one study indicated that [^{3}H]DA binding was decreased in the striatum after chronic antidepressant treatment (Lee and Tang, 1982) DA autoreceptor subsensitivity has also been suggested following biochemical studies (Sc... et al., 1979) and electrophysiological studies (Chiodo and Antelman, 1980). Chronic treatment with a variety of antidepressant drugs has recently been reported to result in a decrease in DA D1 receptor number (Klimek and Nielsen, 1987)

Antidepressant drugs have been widely reported to enhance monoaminergic neurotransmission (Richelson and Pfennig, 1984, Green, 1983, Leinberg, 1987, and result in various changes to monoaminergic receptors and their function However the relationship between the initial inhibition of monoamine uptake and antidepressant activity is not fully understood Most studies investigating the effects of antidepressant drugs are carried out on normal animals or animal tissue but the effect of monoamine uptake inhibition on normally functioning systems may not completely parallel the effect on systems occurring in depressed patients

2.4 THEORIES OF DEPRESSION

Early evidence demonstrating that depletion or inactivation of central NA, for example, after reserpine treatment, caused depression or sedation and that drugs potentiating central NA resulted in excitation and antidepressant effects led to the catecholamine hypothesis of affective disorders (Schildkraut, 1965) This hypothesis proposes that some, if not all,

depressions are associated with an absolute or relative deficiency of catecholamines, particularly NA, at functionally important adrenergic sites in the brain. Conversely an increase in functional brain NA was suggested to be associated with mania, or the manic phase of bipolar depressive disorders (Schildkraut, 1965, Bunney and Garland,1982). The enhancing effect of MAO inhibitors and tricyclic antidepressant drugs on monoaminergic transmission, which has been widely reported, and the evidence of decreased monoamine levels demonstrated in patients, supports this hypothesis. It has been reported that MAO inhibitors and some tricyclic antidepressant drugs affect 5-HT as well as NA in the brain and an indoleamine hypothesis of affective disorders has also been proposed (Murphy et al., 1978, Ögren et al., 1979). This hypothesis suggests that functional brain 5-HT levels are reduced in patients with affective disorders.

However, the effects of antidepressant drugs such as iprindole and mianserin, which do not appear to alter uptake or metabolism of monoamines, have demonstrated a weakness in these hypotheses The effects of the monoamine uptake inhibitors, cocaine and amphetamine, are also in contrast with the catecholamine or indoleamine theories of affective disorders as they have limited use in the long-term treatment of depression (Post, 1978). In addition, the clinical effects of antidepressant drugs do not appear to be consistent with the catecholamine hypothesis as improvement of symptoms is generally evident only after 2-3 weeks of treatment whereas monoamine uptake inhibition by tricyclic antidepressant drugs and MAO inhibition takes place within hours.

Chronic treatment with antidepressant drugs has been reported to cause decreases in β-adrenoceptors and 5-HT_2 receptors (Green, 1988; Manji and Brown, 1988; Syvälanti, 1987) It was therefore suggested that there may be a state of monoaminergic hyperactivity in depression which is corrected by the down-regulation of these receptors (Siever and Sulser, 1984; Lak et al., 1982). More recently, however, it has been proposed that there may be a relative failure in the regulation of neurotransmitter mechanisms in affective disorders rather

than absolute increases or decreases in their activity. This has resulted in the "dysregulation hypothesis of affective disorders" which proposes that the activity of the neurotransmitter system may be highly variable, unstable, inappropriately responsive to incoming information and desynchronized from normal periodicities (Siever and Davis, 1985) Antidepressants have been suggested to be effective in a dysregulated system by re-equilibrating and restoring the system to optimal efficiency.

There has also been evidence that central cholinergic factors may play a role in the aetiology of affective disorders The anticholinesterase, physostigmine, and other agents which increase cholinergic activity in the brain have been reported to exacerbate symptoms in depressed patients. Physostigmine, when administered to patients with the primary affective disorder, mania, caused a rapid decrease in manic symptoms and resulted in changes in mood and thought toward depression (Davis et al., 1978; Janowsky et al., 1973). These results have suggested an adrenergic-cholinergic imbalance in affective disorders with mania being the result of adrenergic predominance and depression being the result of cholinergic predominance (Janowsky et al., 1972)

The role of DA in depression has been implicated by various studies. One of the symptoms of depression is an inability to experience pleasure It has therefore been proposed that reward mechanisms may be hypoactive in depression (Spyraki et al., 1982, Wise, 1978, Willner, 1983) Rewarding effects of stimulant drugs and intracranial self-stimulation have been reported to be mediated by the mesolimbic dopaminergic system (Willner, 1983, Spyraki et al., 1982; Mora and Ferrer, 1986). In support of these proposals, intracranial self-stimulation has been observed when electrodes are placed in the VTA, which resulted in increased metabolism of DA in the nucleus accumbens (Fibiger et al., 1987). In another study, the rewarding effect of MFB stimulation was shown to be attenuated or abolished by neuroleptics (Gallistel, 1986). It has also been demonstrated that chronic treatment with antidepressant drugs potentiates the apomorphine induced locomotion in rats. This effect was reported to be blocked by the DA D2

receptor antagonist haloperidol indicating DA D2 receptor involvement in this effect (Smailowski, 1987). However, dopaminergic hyperactivity may not be the primary dysfunction in depression and these effects may be secondary to other effects (Willner, 1983).

There appears to be a complex interplay between monoaminergic systems in affective disorders and a single mechanism of action of antidepressant treatment seems unlikely. In order to increase the understanding of the aetiology of depression, the distribution and possible interactions of monoamines in the nucleus accumbens of the rat was therefore investigated. DA receptors in different areas of the nucleus accumbens and the effects of antidepressant treatment on them was also studied.

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Animals

Male Wistar rats weighing 230-280g when sacrificed were used for HPLC and receptor studies. For lesion studies male Wistar rats weighing 260-300g were used. Rats were housed five to a cage under standard laboratory conditions with a controlled 12 hour light-dark cycle for at least 2 weeks before experimentation The rats were given free access to Epol food pellets and tap water. In certain experiments the antidepressant drug, desipramine (20mg/kg/day) was administered to the rats in the drinking water (approximately 10mg desipramine/100ml).

3.1.2 Chemicals

All chemicals used were of the highest grade commercially available. Suppliers of these are listed in Appendix A

3.1.3 Instruments

All equipment used during the course of this investigation is listed in Appendix B.

3.2 METHODS

3.2.1 Dissection

A dissection technique was developed which enabled the investigation of at least six discrete

areas within the nucleus accumbens and eighteen areas within the striatum Male Wistar rats weighing 250-280g were sacrificed by decapitation and the brains rapidly removed, chilled on ice and then sliced coronally with a McIlwain tissue chopper set at 0.9mm. Care was taken to ensure that the rat weights did not lie outside the defined limits as slight variations in the dimensions of the brain were observed to affect the cutting co-ordinates of the blade. The slices were placed on a glass slide over ice against a black background to facilitate the definition of the brain areas. The whole nucleus accumbens or dorsal and ventral regions of this nucleus were dissected out of the brain slices approximately corresponding to the anterior posterior co-ordinates, 10 2-9.6, 9.6-9.0; 9.0-8.4 according to the stereotaxic atlas of Pellegrino et al., (1979). Dorsolateral, dorsomedial, ventrolateral and ventromedial areas of the striatum were dissected out of the brain slices approximately corresponding to the anterior posterior co-ordinates 9 6-9.0; 9.0-8.4; 8.4-7.8; 7.8-7 0, and 7 0-6.2 The frontal cortex, hypothalamus and hippocampus were dissected out using a blade or pair of forceps. The tissue was rapidly transferred to liquid nitrogen and stored at -100^{0} C (Figs. 3.1 and 3.2)

3.2.2 Brain lesions

LC lesions were performed by Mr A.H. Engelbrecht and MFB lesions were performed by Mr. A. Jaffer

Rats were anaesthetized with pentobarbitone (50m/kg) and Innovar Vet (0.16ml/kg) The head of the rat was then positioned in a stereotaxic apparatus and aligned according to the co-ordinate system of Pellegrino et al., (1979), with the zero line taken as the inter-aural line and the incisor bar 5mm above the inter-aural line. The head was shaved and disinfected with hibitane (0.5% in 70 % alcohol) before surgery. After exposing the cranium an electric drill was used to make bilateral holes of about 1mm in diameter. The co-ordinates AP = 1.4; ML = 0.8, depth = 6.2mm were used for the LC lesion, and AP = 5.6; ML = 1.6; depth = 9mm for

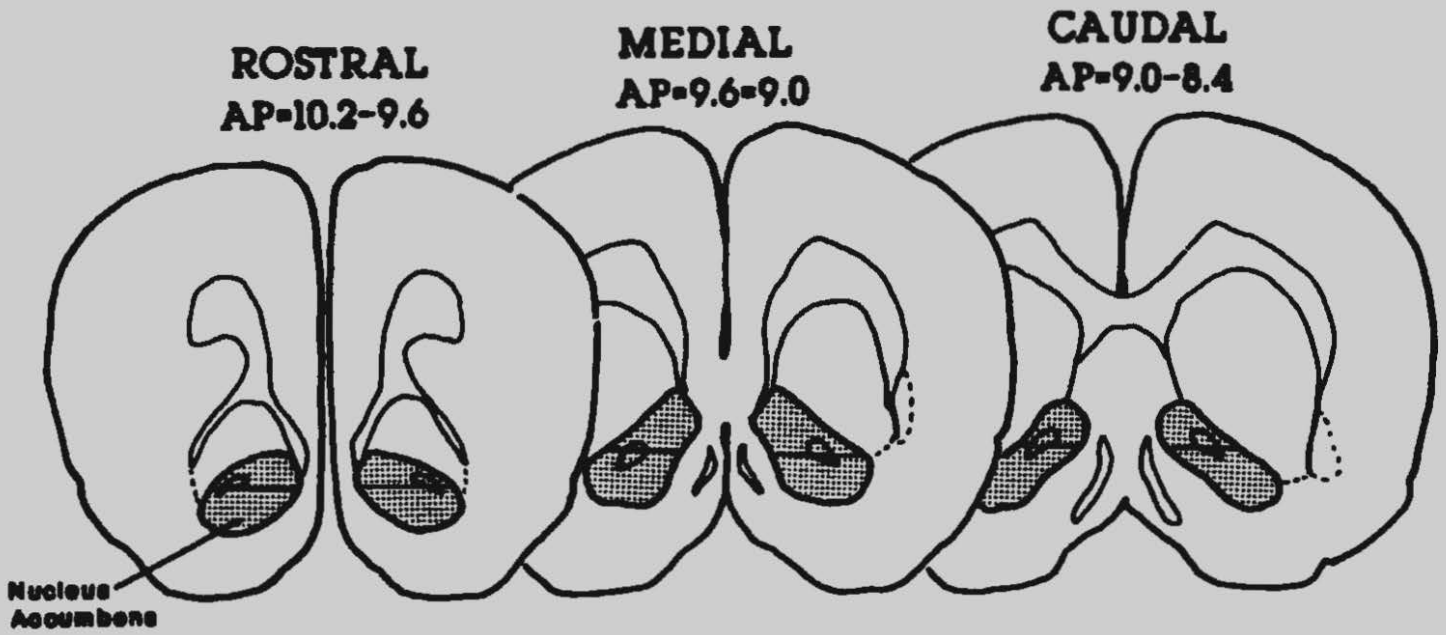

Fig. 8.1 Coronal slices of the rat brain showing the nucleus accumbens.

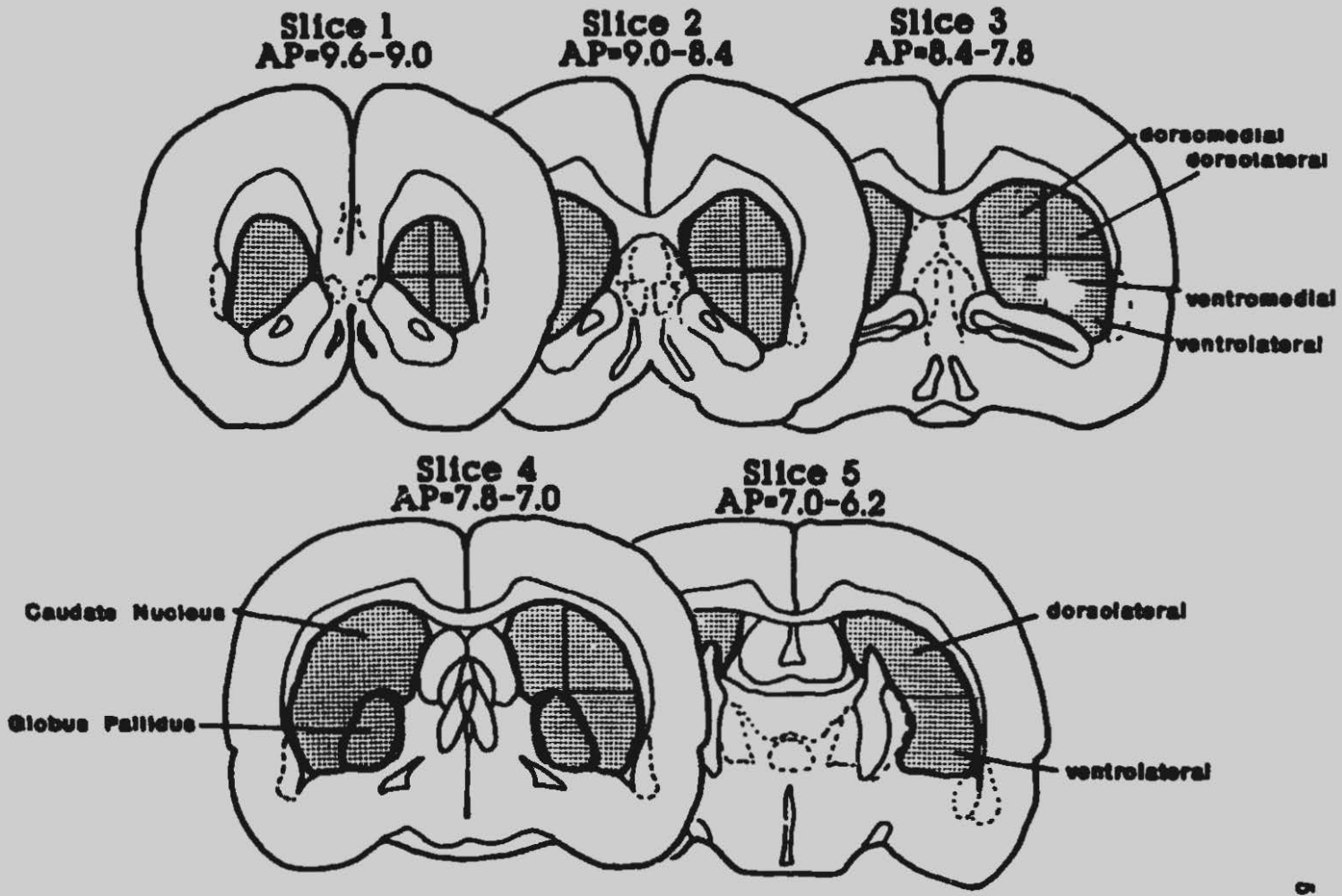

Fig. 3.2 Coronal slices of the rat brain showing the striatum.

the MFB lesion The needle was lowered onto the dura and the vertical co-ordinate noted The depth to which the needle must descend was calculated from this value Rats were injected bilaterally with 1µl saline containing 1mg/ml ascorbic acid for controls or 10mg/ml 6-OHDA in saline containing 1mg/ml ascorbic acid for lesioned rats Two weeks after the lesion the rats were sacrificed by decapitation and the nucleus accumbens, frontal cortex, hypothalamus and hippocampus were dissected out as described in Section 3 3 1. Histology confirmed the site of lesion and HPLC analysis of the frontal cortex, hippocampus and hypothalamus established the effectiveness of the lesion (Fig 3.3)

3.2.3 High performance liquid chromatography

The monoamines NA, DA and 5-HT and the metabolites DOPAC, HVA and 5-HIAA were determined by HPLC with electrochemical detection A 10 x 4.6mm Biophase ODS column (C18 reverse phase, particle size 5m) was used at room temperature (22 ^{0}C). Detection was by a glassy carbon electrode set at +0.70 V versus a Ag/AgCl reference electrode with a sensitivity of 2 nA/V. The mobile phase consisted of 0.1M sodium formate buffer (pH 3 85), 0 5mM ethylenediamine tetra-acetic acid (EDTA), 5mM sodium heptane sulphonic acid, 6% v/v methanol and 4% v/v acetonitrile The flow rate was maintained at 1ml/minute Tissue from individual rats or pooled from 3 rats was weighed, sonicated in 30 volumes of ice cold 0 1M perchloric acid, containing 0.3mM EDTA and 0 5mM sodium metabisulfite, allowed to stand for 20 minutes on ice then centrifuged at 21,000g for 20 minutes (Russell et al., 1985) To enable the NA peak to be better separated from the solvent front, of which a large proportion is ascorbic acid, ascorbic acid oxidase was added to the perchloric acid extracts of the tissue (McKay et al., 1984). It was necessary to adjust the supernatant to pH 5.0 with 10M potassium acetate (5µl/200µl) before the addition of this enzyme Ascorbic acid oxidase (1mg/ml) was dissolved in ammonium acetate buffer (0.1M ammonium acetate adjusted to pH 5 0 with glacial acetic acid) and (5µl/200µl) was added to the extract The samples were allowed to

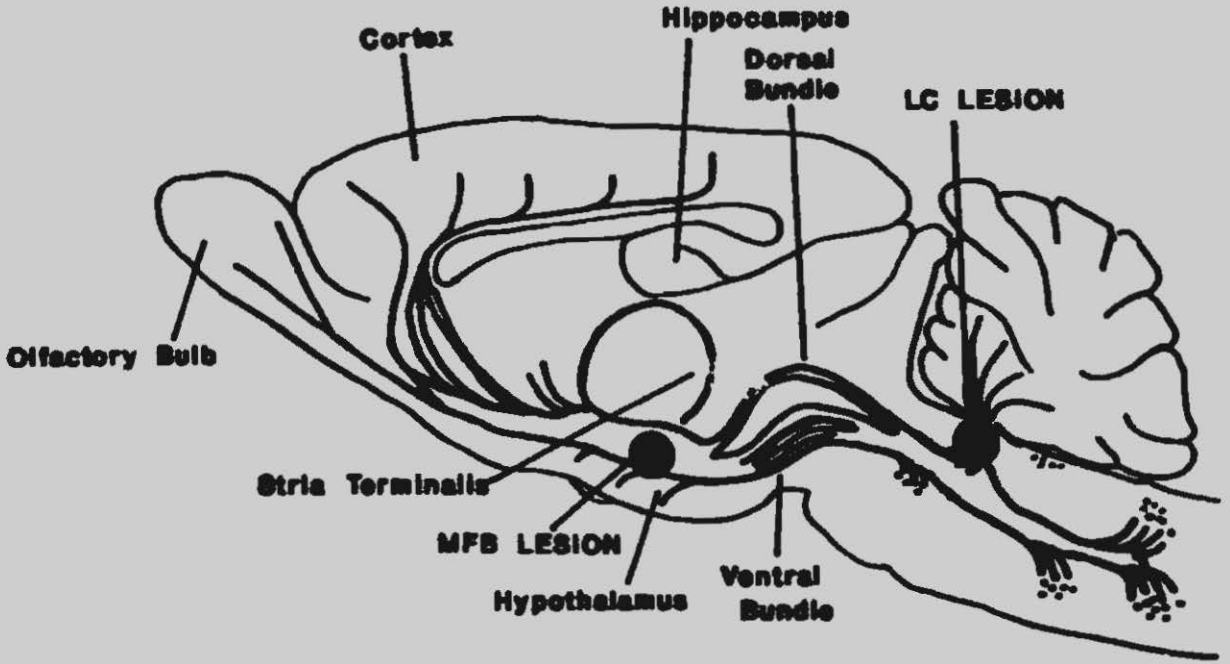

Fig. 8.8 Sagittal projection of the ascending NA pathways showing the positions of the MFB and LC lesions.

stand for 10 minutes on ice then were filtered through a 0.2µm microfilter prior to injection onto the column. Standard solutions of the monoamines and their metabolites were prepared in 0.1M HCl containing 0.1% sodium metabisulfite and were used to quantitate the compounds according to peak height. Twice the peak threshold value for the noise level of the detector was used to determine the limit of sensitivity of the method. The results were expressed as ng/g wet weight of tissue using the following formula:-

$$\text{CONC} = \frac{\text{Sample PH(mV)}}{\text{STD PH(mV)}} \times \frac{[\text{STD}]}{1} \times \frac{\text{Sample Vol(µl)}}{\text{Inject Vol(µl)}} \times \frac{1000}{\text{tissue wt(mg)}}$$

Where CONC is the concentration in ng/g wet wt., [STD] is the concentration of standard in ng/20µl and Inject Vol is the injection volume (20µl).

3.2.4 Purity of Radiochemicals

The purity of the radiochemicals [^{3}H]SCH23390, [^{3}H]spiperone and [^{125}I]iodospiperone was determined by thin layer chromatography on silica gel plates in a solvent mixture of chloroform:methanol (9:1). An SMI pipette fitted with a capillary tube was used to apply 1µl of the radiochemical to the plate which was allowed to elute for approximately 3 hours. Squares (1cm x 1cm) were cut out of the plate, placed in vials with 10ml of scintillation fluid and mixed thoroughly before counting in a liquid scintillation counter. The purity of [^{3}H]SCH23390 when in use was found to be 98.6% and only dropped to 92.3% after 9 months. The purity of [^{3}H]spiperone was between 96.0 and 97.7% and the purity of [^{125}I]iodospiperone was 99.2%

3.2.5 Radioligand Binding

Details of the characterization of radioligand binding can be found in Chapter 6. A brief outline of the final method adopted is described here. Tissue was thawed and homogenized in a polycarbonate test-tube using a teflon pestle, with clearance of 0.9mm, attached to a stirrer set at 900 rpm in 40 volumes of ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and 1µ M pargyline (pH 7.4 at 25° C). The homogenate was centrifuged at 35,000g for 10 minutes and subsequently washed twice with 40 volumes of buffer, then resuspended in sufficient buffer to give 0.05mg protein/ml for [^{3}H]SCH23390 binding and 0.15mg protein/ml for [^{3}H] spiperone binding in the final assay volume (Leysen and Gommeren, 1981).

Polypropylene incubation tubes (in duplicate) received 20µ l of the radioligand, with or without the displacing drug (1µ M) cis-flupenthixol for [^{3}H]SCH23390, or 10µM sulpiride for [^{3}H]spiperone) to define nonspecific binding. Ketanserin (30nM or 3nM) was added throughout to prevent the radioligand binding to $5\text{-}HT_2$ receptors. Four hundred microlitres of tissue suspension was added, and the tubes were incubated at 37°C for 30 or 15 minutes, respectively, for DA D1 and D2 receptor assays. The reaction was terminated by the addition of 5ml of ice cold buffer to each tube followed by rapid vacuum filtration through Schlecher and Schuell No 3362 filters previously soaked in 0.025% Brij. Filters were washed twice with 5ml of buffer and the radioactivity was determined by liquid scintillation counting after the addition of 10ml Scintillator 299 (Packard). Protein concentration was measured by the method of Lowry et al. (1951).

3.2.6 Analysis of Radioligand Binding Data

Saturation binding experiments (Fig. 3.4) are one of the most commonly performed equilibrium experiments and a variety of different methods have been used to calculate

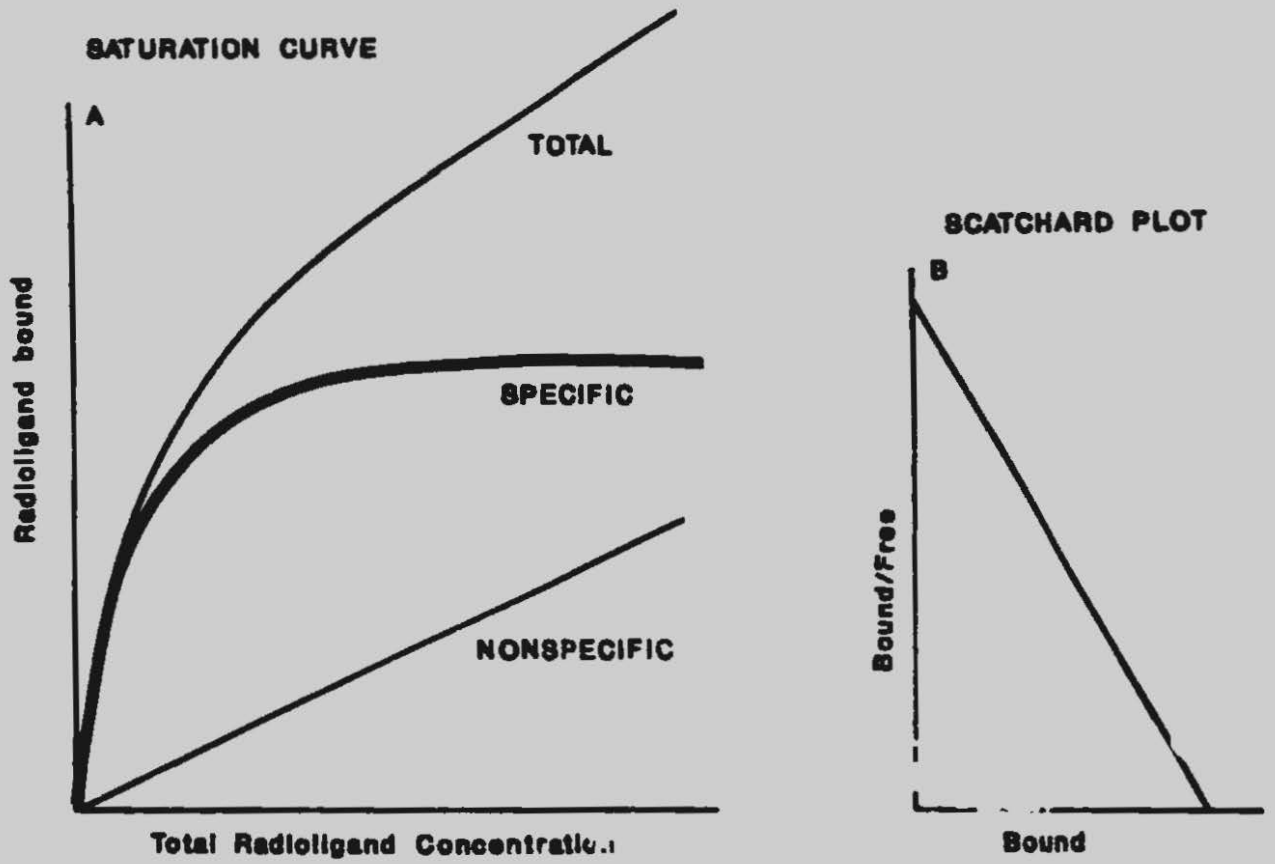

Fig. 3.4 Diagrammatic representation of a SATURATION CURVE (A) and a SCATCHARD PLOT (B).

binding parameters from the data obtained. In saturation experiments increasing concentrations of radioactively labelled ligand are added to a constant concentration of receptors in a preparation. At equilibrium the free labelled ligand is washed from the preparation and the total amount of labelled ligand bound is determined. Nonspecific binding is measured in the presence of excess unlabeled ligand and specific binding is determined by subtraction of nonspecific from total binding. From the binding isotherm (Fig. 3.4) the total binding capacity of the saturable receptor (Bmax) is shown by the horizontal asymptote and the equilibrium dissociation constant (K_D) is defined by the free ligand concentration at half-saturation or which occupies half of the total receptors. Scatchard, (1949) introduced a simple method of calculating the Bmax and K_D by transformation of the data to give a linear relationship. This method is analogous to that developed by Eadie and Hofstee for enzyme kinetics, and involves plotting [Bound]/[Free] versus [Bound] where the intercept on the abscissa (B/F = 0) is the Bmax and the negative reciprocal of the slope is the K_D. Other methods which have been used involve plotting 1/[Bound] vs 1/[Free] (Lineweaver-Burk plot) and [Free/Bound] vs [Free] (Woolf plot). However transformations such as the Scatchard plot have various limitations and have been shown (Bürgisser, 1984) to lead to inaccurate estimates of binding parameters. For example too low a radioligand concentration causing clustering of the data points in a small range on the x-axis and distortion of statistical error structure due to the transformation causing increased scatter of data points especially at low radioligand concentrations. In addition the Scatchard plot is unsuitable for linear regression as the required statistical assumptions are not met, both the x- and y-variables being dependent on prior calculation. Computerized nonlinear least squares regression analysis is an alternative approach now being widely used which avoids the pitfalls encountered in the transformation methods. Munson and Rodbard (1980) developed such a program called LIGAND. This program uses a weighted least squares curve-fitting algorithm for estimating the binding parameters of any number of ligands reacting simultaneously with any number of receptors. As an exact mathematical model of the ligand binding system is used errors or

biases introduced by transformation of data is avoided The total ligand concentration rather than the free ligand concentration (which is determined by subtraction) is taken as the independent variable as this can be controlled precisely, and the nonspecific binding is taken as an unknown factor which is estimated simultaneously with the other parameters These parameters are forced to assume plausible values and the program adjusts them by iteration until the model approximates the actual data points

3.2.7 Statistical Analysis

Statistical analysis of the data was performed by Dr R. Schall of the MRC Institute for Biostatistics Area differences for monoamines in the nucleus accumbens were analysed using ANOVA (analysis of variance) followed by a paired t test Bonferroni significance was achieved at $P<0.0033$ Area differences for monoamines in the striatum were analysed using ANOVA followed by Tukey's multiple comparison

A non parametric analysis, the sign test, was performed on monoamine ratios in the nucleus accumbens In a pairwise comparison of two areas, the differences between the 10 values in the two areas were computed and the number of like signed differences was counted To achieve Bonferroni significance at $P < 0.0033$ the smaller number of like signed differences must be zero To analyse treatment differences for monoamines in the nucleus accumbens between LC and MFB lesioned rats and their controls the Mann Whitney non parametric test was used

Differences between saline and DSP4 treated rats were analysed for each area separately using the t test.

ANOVA and Tukey's multiple comparison test were used to determine whether significant differences existed in receptor number and affinity among areas of the nucleus accumbens

Differences in DA D1 and D2 receptor number and affinity due to treatment with desipramine were analysed using a paired t test

CHAPTER 4

REGIONAL DISTRIBUTION OF MONOAMINES IN THE NUCLEUS ACCUMBENS AND STRIATUM

4.1 INTRODUCTION

Depression has been suggested to be associated with noradrenergic dysregulation (Richelson and Pfenning, 1984, Schildkraut, 1965), serotonergic hypoactivity (Sulser and Mishra, 1983, van Praag, 1982, Murphy et al., 1978), as well as hypoactivity of a reward system in which the mesolimbic dopaminergic neurons are involved (Fibiger and Phillips, 1981, Spyraki et al., 1982, Willner, 1983, Chapter 2) The nucleus accumbens is a major terminal area for these mesolimbic dopaminergic neurons (Oades and Halliday, 1987, Ungerstedt, 1971a, Lindvall and Stenevi, 1978, Chronister et al., 1980) which arise in the VTA of the midbrain (Section 1.2.2) The nucleus accumbens has also been reported to be innervated by serotonergic (Azmitia and Segal, 1978, Section 1 2 3) and noradrenergic neurons (Lindvall and Stenevi, 1978, O'Donohue et al., 1979; Lindvall and Björklund, 1974; Swanson and Hartman, 1975, Section 1 2 1) The nucleus accumbens is not a homogeneously organized structure (Chronister et al., 1980, Herkenham et al., 1984, de France et al., 1983) and interactions between noradrenergic and dopaminergic (Nurse et al., 1984), serotonergic and noradrenergic (Manier et al., 1987) and serotonergic and dopaminergic systems (Nurse et al., 1988) have been reported to occur in this terminal area A detailed investigation of the distribution of monoamines within the nucleus accumbens has not previously been carried out and therefore in order to determine whether there was any overlap between the various monoamine terminals in the nucleus accumbens, NA, DA, 5-HT and their metabolites DOPAC, HVA and 5-HIAA were measured in six areas of the nucleus accumbens

The striatum is a major terminal area for the nigrostriatal dopaminergic neurons (Fillous et

al., 1987a; Jimenez-Castellanos and Graybiel, 1987, Section 1 2.2) and it has also been shown to be a heterogeneously organized structure (Joyce et al., 1986, Doucet et al., 1986, Tassin et al., 1976, Beal and Martin, 1985). Although some overlap has been shown to exist between the striatum and the nucleus accumbens, these nuclei have been accepted to be separate (Walsh et al., 1981) The striatum has been reported to receive negligible (Doucet et al., 1986, Jones and Moore, 1977) or very little (O'Donohue et al., 1979; Lindvall and Bjorklund, 1974) innervation by noradrenergic neurons but has been shown to receive substantial serotonergic input from the dorsal raphe (Azmitia, and Segal, 1978, Soghomonian et al., 1987, Steinbusch et al., 1981, Section 1 2 3) Monoamine and metabolite concentrations were therefore determined in eighteen areas of the striatum so that a detailed study of their distribution could be made and compared to that of the nucleus accumbens

4.2 METHODS

Male Wistar rats weighing 250-280g were sacrificed by decapitation, and the brains rapidly removed and placed on ice The nucleus accumbens was dissected into 6 areas and the striatum into 18 areas as described in Section 3 2 1 Perchloric acid extracts of tissue from individual rats for the nucleus accumbens or pooled from 3 rats for the striatum were analysed by HPLC with electrochemical detection as described in Section 3 2 3

4.3 RESULTS

4.3.1 Distribution of monoamines in the nucleus accumbens

DA concentrations were found to be significantly lower in the rostral than in the medial and caudal slices of the nucleus accumbens The concentration in the ventrorostral area was significantly lower than in the dorsorostral area There was a similar distribution of DOPAC in

the nucleus accumbens, being significantly lower in the rostral slice than in the medial and caudal slices. HVA followed a similar pattern (Table 4.1, Fig 4.1) DA levels were higher than NA levels throughout the nucleus accumbens DA/NA ratios ranged from 3.9 to 40 (Table 4.1). There also appeared to be regional heterogeneity in the NA concentration (Fig. 4.2) NA levels were significantly higher in the caudal slice than in the rostral and medial slices. The concentration in the dorsomedial area was significantly lower than the concentration in the ventrorostral and dorsorostral areas No significant differences were observed between ventral and dorsal concentrations of NA 5-HT and 5-HIAA levels were lower than DA levels and the ratio of DA/5-HT ranged from 2.1 to 18.8 The 5-HT concentration was highest in the ventrocaudal, dorsocaudal and ventromedial areas and lowest in the dorsorostral, ventrorostral and dorsomedial areas (Fig 4.3)

4.3.2 Metabolite/monoamine ratios in different areas of the nucleus accumbens

The metabolite/monoamine ratios were calculated and DOPAC/DA, HVA/DA, HVA/DOPAC and 5-HIAA/5-HT ratios are shown in Table 4.2 Significant differences were observed in the DOPAC/DA and HVA/DA ratios, being highest in the rostral slice and lowest in the caudal slice There were also significant differences in the 5-HIAA/5 HT ratios, being highest in the dorsorostral, ventrorostral and dorsomedial areas, and similar in the ventromedial, dorsocaudal and ventrocaudal areas.

4.3.3 Distribution of monoamines in the striatum

The DA concentration of the dorsal part of slice 1 and the whole of slice 2 was significantly higher than the DA concentration in the dorsal or the ventral areas of the most caudal slice (Table 4.3, Fig 4.4) The dorsomedial DA concentration decreased along the rostrocaudal axis The DA concentration in the lateral areas increased from the most rostral slice to the following

TABLE 4.1

Regional distribution of monoamines in the nucleus accumbens of the rat

		Concentration		
Monoamine	Area	Rostral	Medial	Caudal
NA	dorsal	237± 17	165± 13[a,b,c,d]	441± 28[a,e,f]
	ventral	219± 17[g]	210± 26	431± 40[h]
DA	dorsal	2134± 489	6529± 435[a,e,i]	6026± 421[a,h,i]
	ventral	851± 292[d,f,h]	5059± 517[a,g]	7453± 332[a]
DOPAC	dorsal	1609± 314	3473± 246[b,c,e,h,i]	2413± 174[c]
	ventral	849± 172	2698± 177[b]	2647± 201[h]
HVA	dorsal	314± 29	593± 21[a,e,i]	474± 17[a,g]
	ventral	239± 26	549± 37[e]	589± 19[h]
5-HT	dorsal	345± 21	347± 8 2[d,f,i]	606± 39[a,h]
	ventral	407± 18	645± 33[a]	842± 45[a]
5-HIAA	dorsal	492± 29	460± 20[d,f]	530± 26
	ventral	511± 29[g]	550± 19	625± 23[h]

Results are the mean± SEM of 10 observations
Between rat variation was highly significant, $p<0.01$
Paired t-tests were therefore used to make pairwise comparisons between areas of individual rats
To reach Bonferroni significance the p-value must be < 0.0033
[a] Significant difference vs dorsorostral area $p<0.0001$,
[b] vs dorsocaudal $p<0.0001$; [c] vs ventrorostral $p<0.0033$,
[d] vs ventrocaudal $p<0.0001$, [e] vs ventrorostral $p<0.0001$,
[f] vs ventromedial $p<0.0001$, [g] vs ventrocaudal $p<0.0033$,
[h] vs dorsorostral $p<0.0033$, [i] vs ventromedial $p<0.0033$,
[j] vs dorsocaudal $p<0.0033$

slice, then decreased more gradually along the rostrocaudal axis so that whereas rostrally, DA levels were higher medially, caudally they were higher laterally. The DA concentration in the ventromedial area of slice 4, the area corresponding to the globus pallidus, was significantly lower than any other area of the same slice. The concentration in the dorsomedial area of slice 4 was significantly lower than that in the dorsolateral and ventrolateral areas of slice 4. In slices 1 and 2 of the striatum the DA level was found to be one and a half times as high as in the nucleus accumbens of the corresponding slices. These are the slices which contain the highest

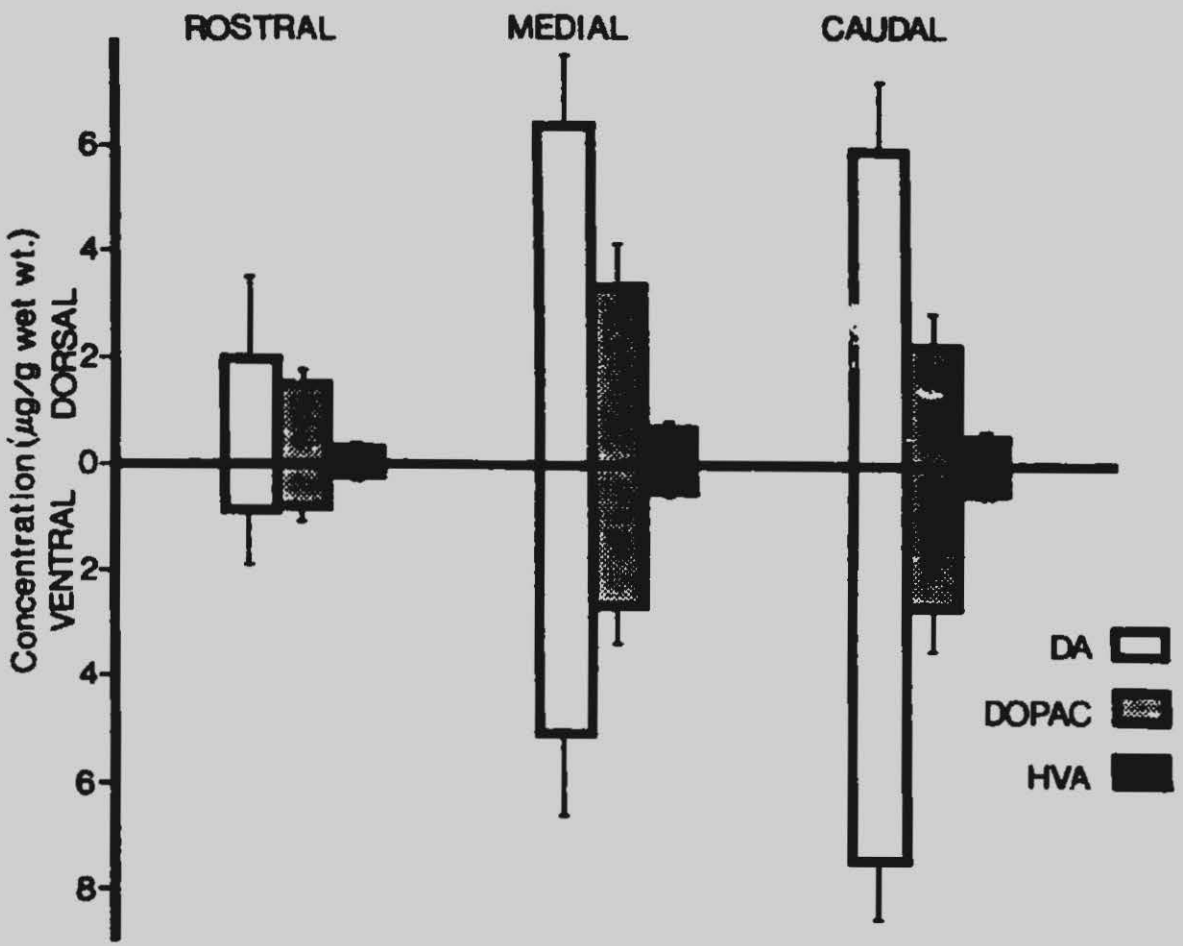

Fig. 4.1 Distribution of DA and its metabolites in the nucleus accumbens of the rat. The mean ± SEM of 10 observations are shown.

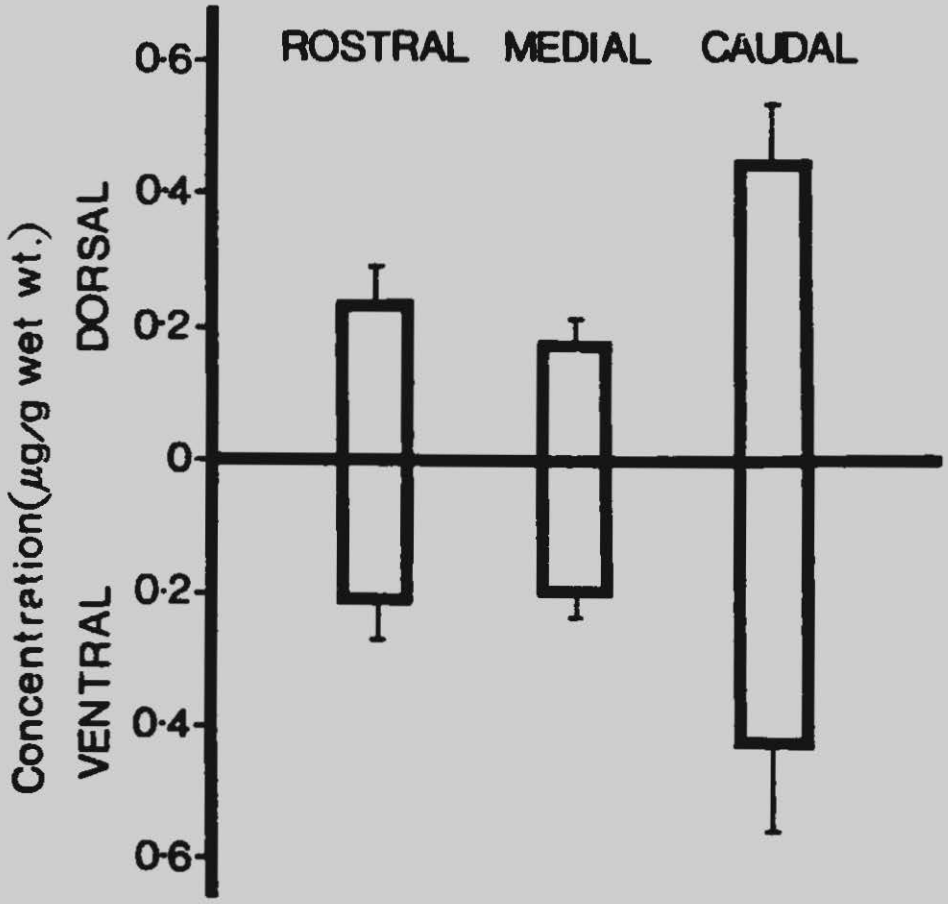

Fig. 4.2 Distribution of NA in the nucleus accumbens of the rat. The mean ± SEM of 10 observations are shown.

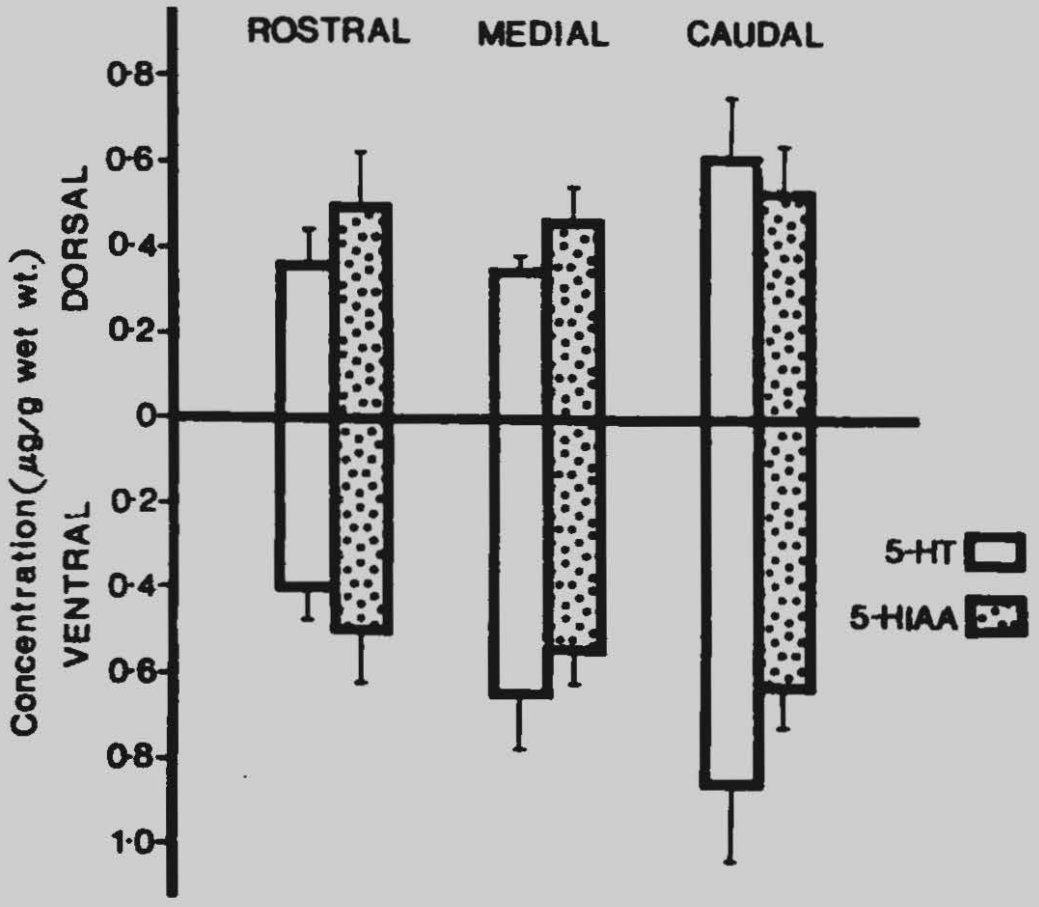

Fig. 4.5 Distribution of 5-HT and its metabolite 5-HIAA in the nucleus accumbens of the rat. The mean ± SEM of 10 observations are shown.

TABLE 4.2

Metabolite/monoamine ratios in different areas of the rat nucleus accumbens.

Metabolite/ monoamine ratio	Area	Rostral	Medial	Caudal
DOPAC/DA	dorsal	0.913± 0.874	0.554± 0.040[a,b]	0.412± 0.023[a,c]
	ventral	1.471± 0.213[b,c,d]	0.554± 0.032[b,c]	0.357± 0.026[c]
HVA/DA	dorsal	0.203± 0.027	0.094± 0.005[a]	0.081± 0.003[a,c]
	ventral	0.679± 0.161[b,c,d]	0.110± 0.005[b]	0.080± 0.004[c]
HVA/DOPAC	dorsal	0.217± 0.012	0.175± 0.077[a,b]	0.205± 0.012
	ventral	0.426± 0.077[c]	0.206± 0.010	0.233± 0.010
5-HIAA/ 5-HT	dorsal	1.491± 0.110	1.343± 0.078[b,d]	0.906± 0.042[a,c]
	ventral	1.286± 0.081[b,d]	0.882± 0.042[c]	0.770± 0.020[c]

Results are the mean± SEM of 10 observations. A non parametric analysis was performed. In a pairwise comparison of 2 areas, the differences between the 10 corresponding values in the two areas were computed, and the number of like signed differences was counted (sign-test). To achieve Bonferroni significance at $p<0.0033$, the smaller number of like signed differences must be zero.

[a] significant difference vs. ventrorostral;[b] vs. ventrocaudal;[c] vs. dorsocaudal;[d] vs. ventromedial.

concentration of DA in both the nucleus accumbens and the striatum. DOPAC levels followed the same pattern as DA levels, being highest in the rostral slice and lowest in the caudal slice. Concentrations of DOPAC were also observed to be lower in the globus pallidus than in any other area of the same slice. HVA levels in the striatum followed a trend similar to DA levels showing an initial increase rostrocaudally followed by decrease which was more rapid in the medial than in the lateral areas. In comparison to DA levels NA levels in the striatum were found to be very low. Excluding the globus pallidus, DA/NA ratios ranged from 19.3 to 369.1. The lowest NA concentrations were found in the dorsomedial and dorsolateral areas where levels were similar in all slices. NA levels in the ventrolateral areas of each slice were also similar, but somewhat higher than the levels in the dorsal areas. NA levels in the ventromedial areas of the striatum increased along the rostrocaudal axis. The NA level was found to be significantly higher in the globus pallidus than in any other area of the same slice.

TABLE 4.3

Regional distribution of monoamines in the striatum of the rat.

		Concentration (ng/g wet wt.)			
Mono amine	Slice	Ventromedial	Ventrolateral	Dorsomedial	Dorsolateral
NA	1	30± 3 3[a,b]	88± 29[a,b]	30± 2 9[a,b]	32± 2 7[a,b]
	2	65± 14[a,b]	70± 10[a,b]	40± 13[a,b]	34± 5 0[a,b]
	3	127± 19[a]	63± 9 2[a,b]	35± 4 4[a,b]	44± 8 6[a,b]
	4	261± 69	103± 18[a]	54± 11[a,b]	44± 14[a,b]
	5		213± 50		50± 11[a,b]
DA	1	8451± 1984[b,c]	6946± 308[a,d]	11036± 1127[a,c,d]	9463± 1108[b,c]
	2	9257± 754[b,c]	8722± 989[b,c]	9278± 818[b,c]	9565± 965[b,c]
	3	6837± 648[a]	10244± 737[b,c]	6804± 481[a]	8293± 581[b,c]
	4	1598± 282[f]	7396± 472[a]	3718± 588	6635± 611
	5		4165± 494		4585± 415
DOPAC	1	3156± 163[a]	2357± 209[a,b]	2556± 245[a,b,c]	1921± 216[a]
	2	2497± 151[b,c,d]	2179± 140[c,d]	2230± 180[c,d]	1835± 186[a]
	3	2082± 197[c]	2192± 155[c,d]	1851± 219[a]	1800± 148[a]
	4	692± 77[b,f]	1824± 108	1301± 81	1700± 80
	5		1516± 177		1336± 75
HVA	1	788± 100[a,b,c]	727± 88[a,b]	635± 74[a]	678± 74[a,b]
	2	800± 41[a,b,c]	866± 32[a,b,c,d]	595± 42[a]	742± 50[a,b]
	3	622± 47[a,b]	989± 127[a]	524± 62[a]	713± 65[a,b]
	4	298± 21[f]	704± 32[a,b]	387± 47	604± 48
	5		439± 39		416± 25
5-HT	1	241± 27[b,c,e]	312± 27[a,e]	167± 15[b,c,d,e]	193± 20[b,c,e]
	2	470± 58[a,c]	483± 69[a,c,e]	261± 22[b,c,e]	242± 27[b,c,e]
	3	751± 80[b,c,d,e]	579± 72	409± 35[a,b,c]	368± 45[a,b,c]
	4	768± 50[b,e]	734± 57[a]	401± 64[b]	393± 36[b]
	5		697± 95		524± 33
5-HIAA	1	370± 28[b,c,e]	408± 26[b,c,e]	305± 17[b,c,d,e]	303± 8.4[b,c,d,e]
	2	500± 46[a,c]	512± 38[a,c]	327± 8 6[b,c,e]	352± 21[b,c,e]
	3	786± 52[a,b,c,d]	670± 64[a,b,c]	418± 25[a,b,c]	473± 62[a,b,c]
	4	989± 41[b,c,e]	782± 32[b,c,e]	504± 36[b]	502± 26[b]
	5		837± 75[f]		594± 43

Results are the means± SEM of 5 observations
A two way ANOVA model was fitted to each of the 6 data sets with factors RAT and AREA. For each monoamine the factor RAT was significant thus the 5 observations for each area were paired with the corresponding observations in the other areas. Area differences for monoamines were analysed using Tukey's studentized range test shown in Appendix C 1 ($P<0.05$) * Mean± SEM of 4 observations.

[a] Significant difference vs ventromedial, slice 4,

[b] vs ventrolateral, slice 5;
[c] vs ventro- and dorsomedial, slice 4;
[d] vs dorsomedial, slice 1;
[e] vs ventro- and dorsomedial, slice 3, dorsolateral, slice 4 and dorsolateral, slice 5;
[f] vs ventro- and dorsolateral, slice 4;
[g] vs all areas except dorsomedial, slice 1 and ventromedial, slice 2;
[h] vs ventro- and dorsolateral, slice 5;
[i] vs dorsolateral, slice 4;
[j] vs dorsolateral, slice 5;
[k] vs dorsomedial, slice 3;
[l] vs dorsomedial, slices 1, 2 and 3;
[m] vs all areas except ventromedial, slices 1 and 2 and ventrolateral, slice 2;
[n] vs ventromedial, slices 3 and 4 and ventrolateral, slices 4 and 5,
[o] vs ventromedial, slice 1 and ventrolateral, slices 2 and 3;
[p] vs ventrolateral, slice 3;
[q] vs dorsomedial and dorsolateral, slice 4;
[r] vs dorsolateral, slice 2;
[s] vs dorsomedial, slice 2;
[t] vs dorsolateral, slice 3;
[u] vs ventrolateral, slice 4,
[v] vs ventromedial and ventrolateral, slice 2.

Similarly, in slice 3, the NA level was significantly higher in the ventromedial area than in any other area of the same slice (Table 4.3, Fig. 4.5). The concentration of 5-HT in the striatum was significantly higher in the ventral than in the dorsal areas of all rostrocaudal slices (Table 4.3, Fig. 4.6), levels were found to be similar in medial and lateral parts of dorsal or ventral areas of each slice. Concentrations of 5-HT were observed to increase along the rostrocaudal axis. 5-HT levels were lower than DA levels in the striatum and excluding the globus pallidus DA/5-HT ratios ranged from 6.0 to 80.4. 5-HIAA levels showed a similar pattern to 5-HT being significantly higher ventrally than dorsally and increasing along the rostrocaudal axis. Fig. 4.7 to 4.12 show the distribution of monoamines in the striatum as median values with their ranges indicated.

4.3.4 Metabolite/monoamine ratios in different areas of the striatum

The ratios of DOPAC/DA, HVA/DA, HVA/DOPAC and 5-HIAA/5-HT in the striatum were calculated (Table 4.4) and in contrast to the nucleus accumbens, no significant differences were observed in DOPAC/DA, HVA/DA or 5-HIAA/5-HT ratios throughout the striatum. The

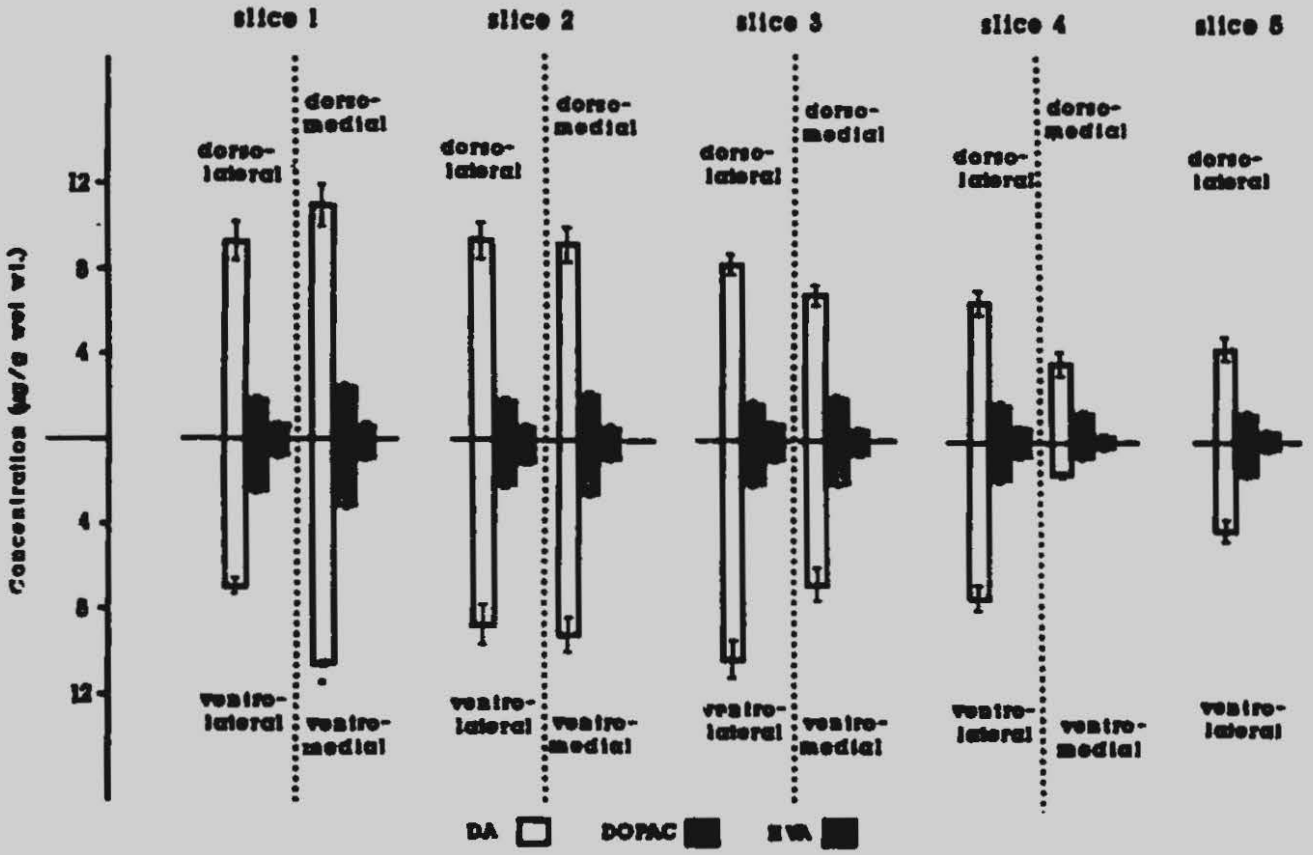

Fig. 4.4 Distribution of DA and its metabolites in the striatum of the rat. The mean ± SEM of 5 observations are shown.
* Mean ± SEM of 4 observations

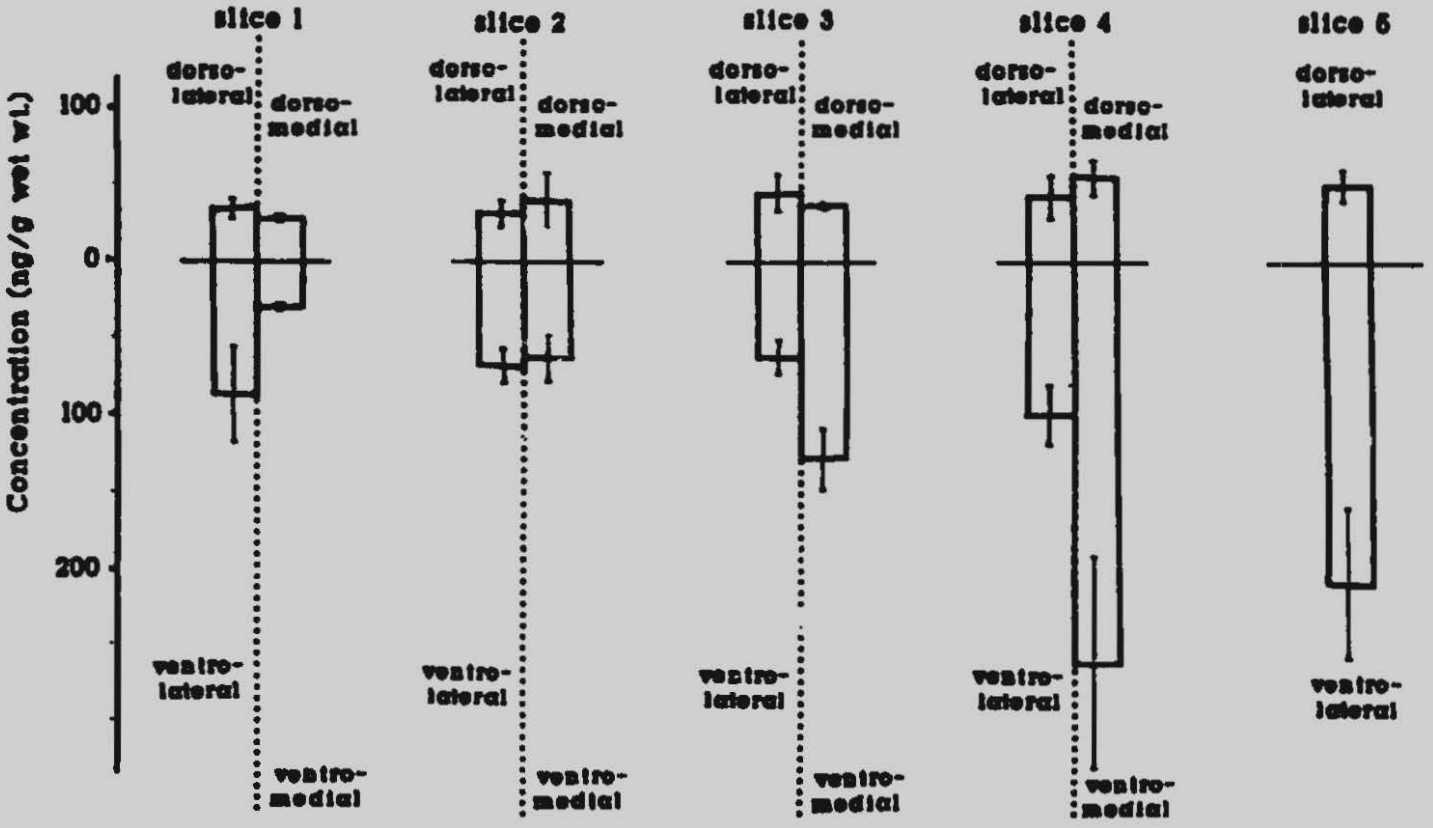

Fig. 4.6 Distribution of NA in the striatum of the rat. The mean ± SEM of 6 observations are shown.

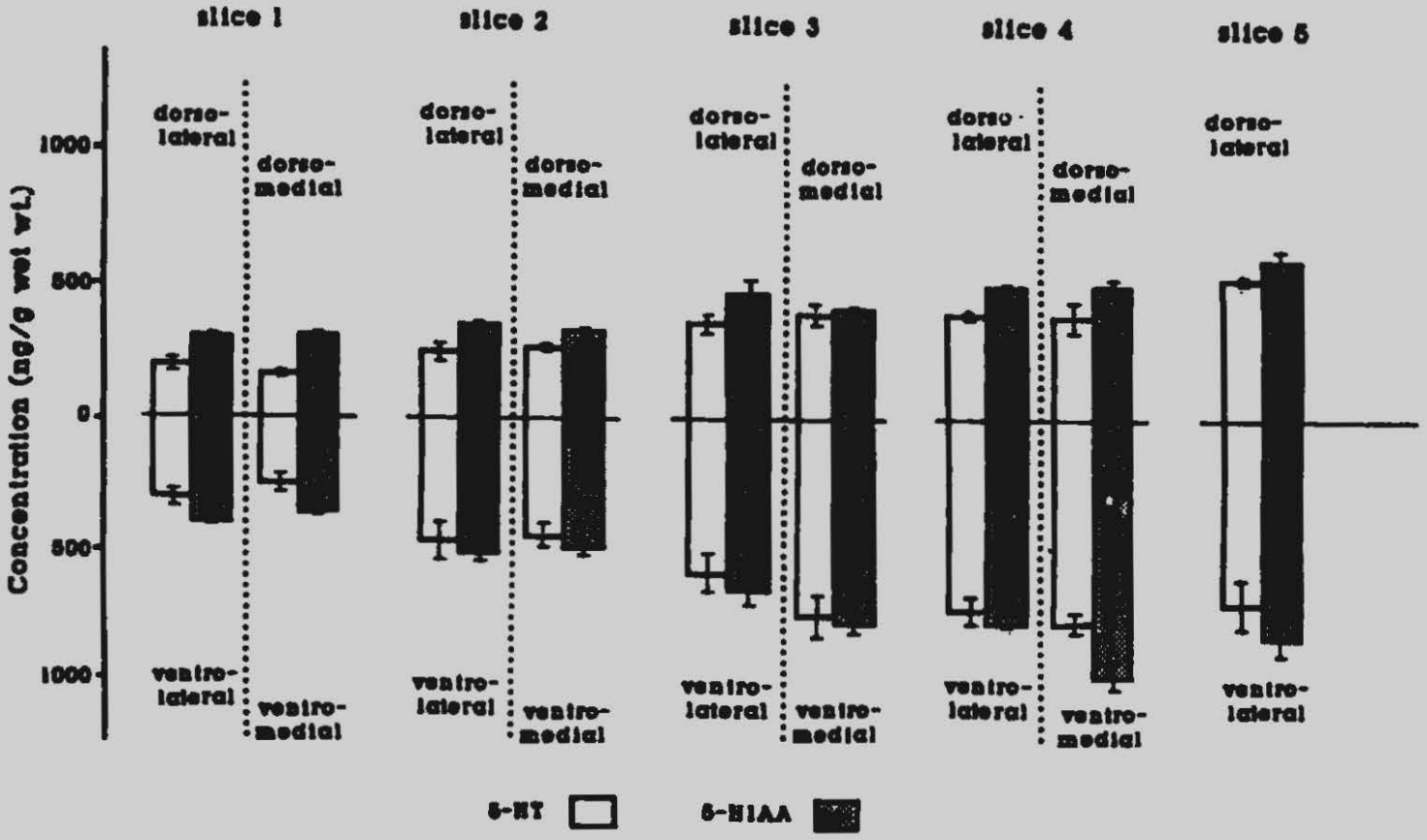

Fig. 4.8 Distribution of 5-HT and its metabolite 5-HIAA in the striatum of the rat. The mean ± SEM of 5 observations are shown.

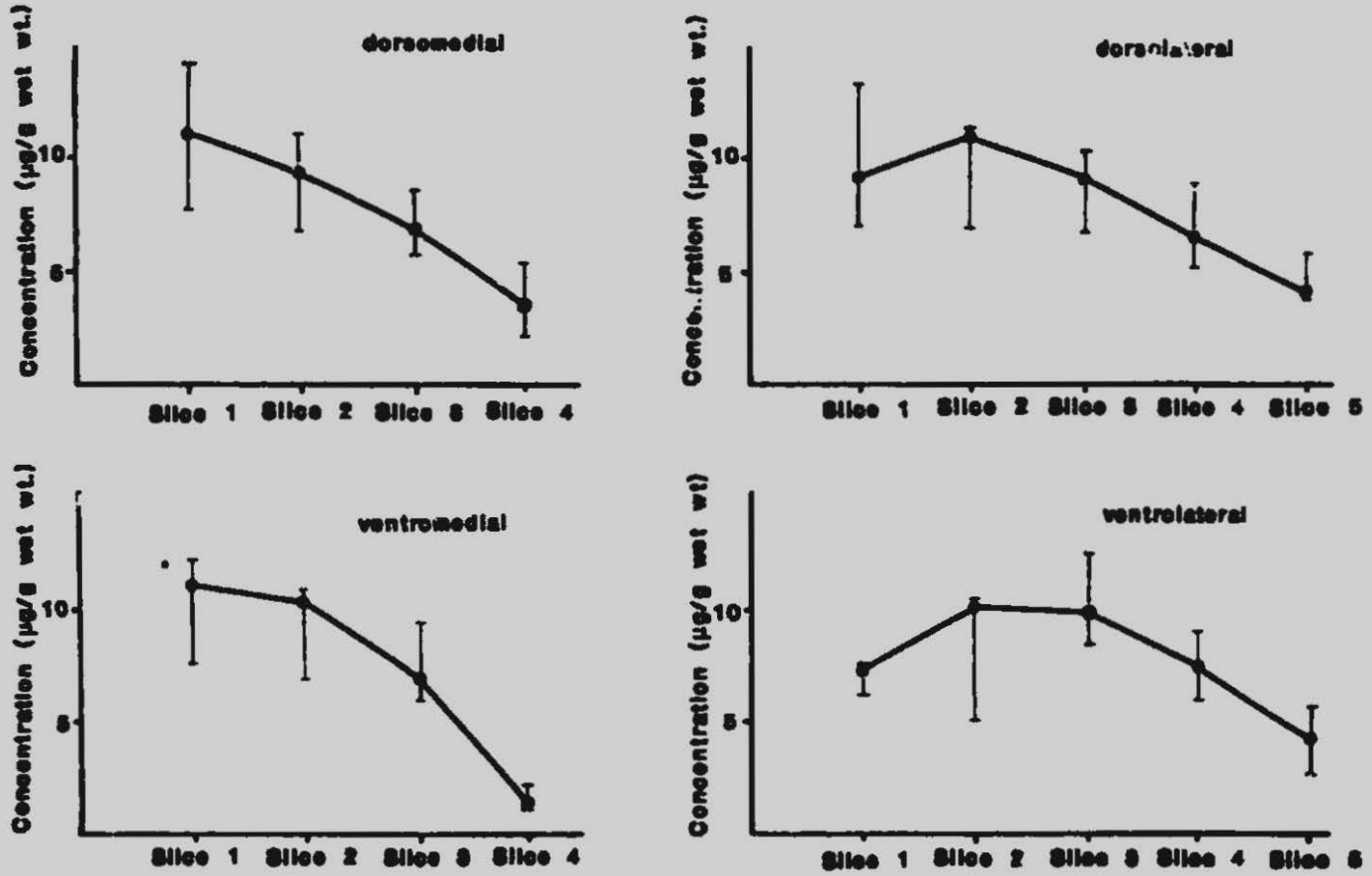

Fig. 4.7 Distribution of DA in quadrants of 5 coronal slices of the rat brain containing the striatum as depicted in Fig. 8.2. The medians of 5 observations with their ranges are shown.
• Median and range of 4 observations.

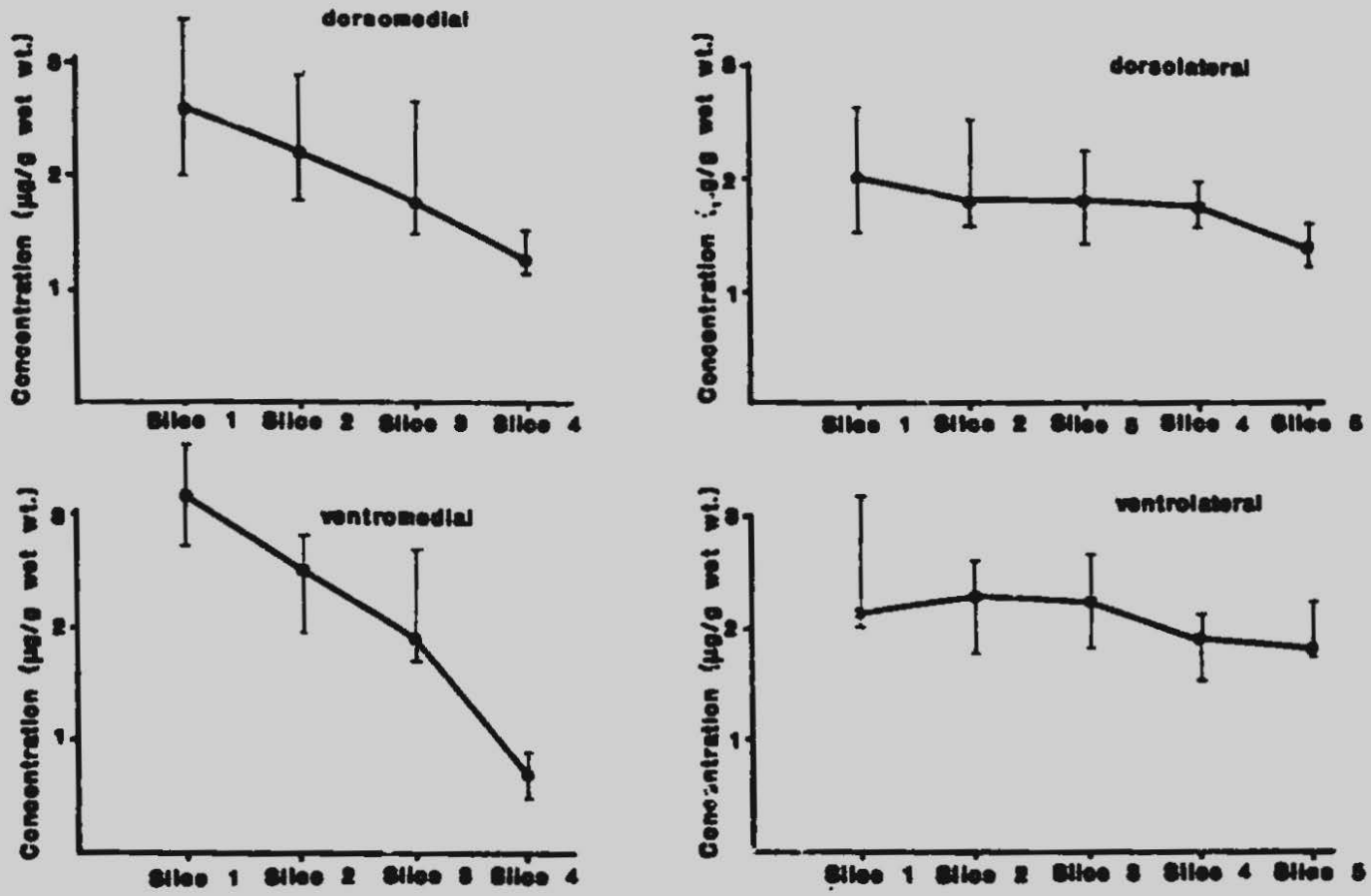

Fig. 4.8 Distribution of DOPAC in quadrants of 5 coronal slices of the rat brain containing the striatum as depicted in Fig. 3.2. The medians of 5 observations with their ranges are shown.

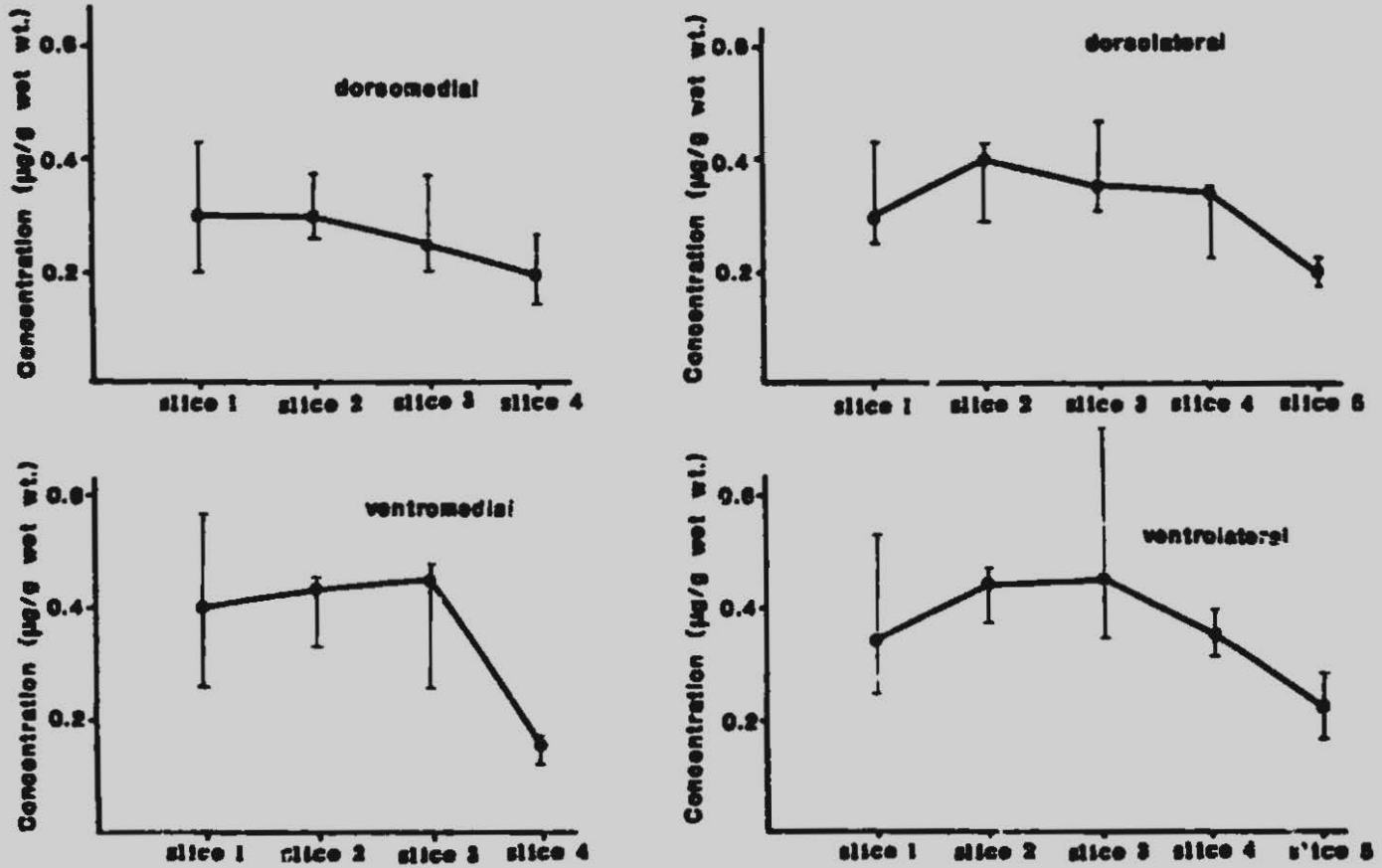

Fig. 4.9 Distribution of HVA in quadrants of 5 coronal slices of the rat brain containing the striatum as depicted in Fig. 8.2. The medians of 5 observations with their ranges are shown.

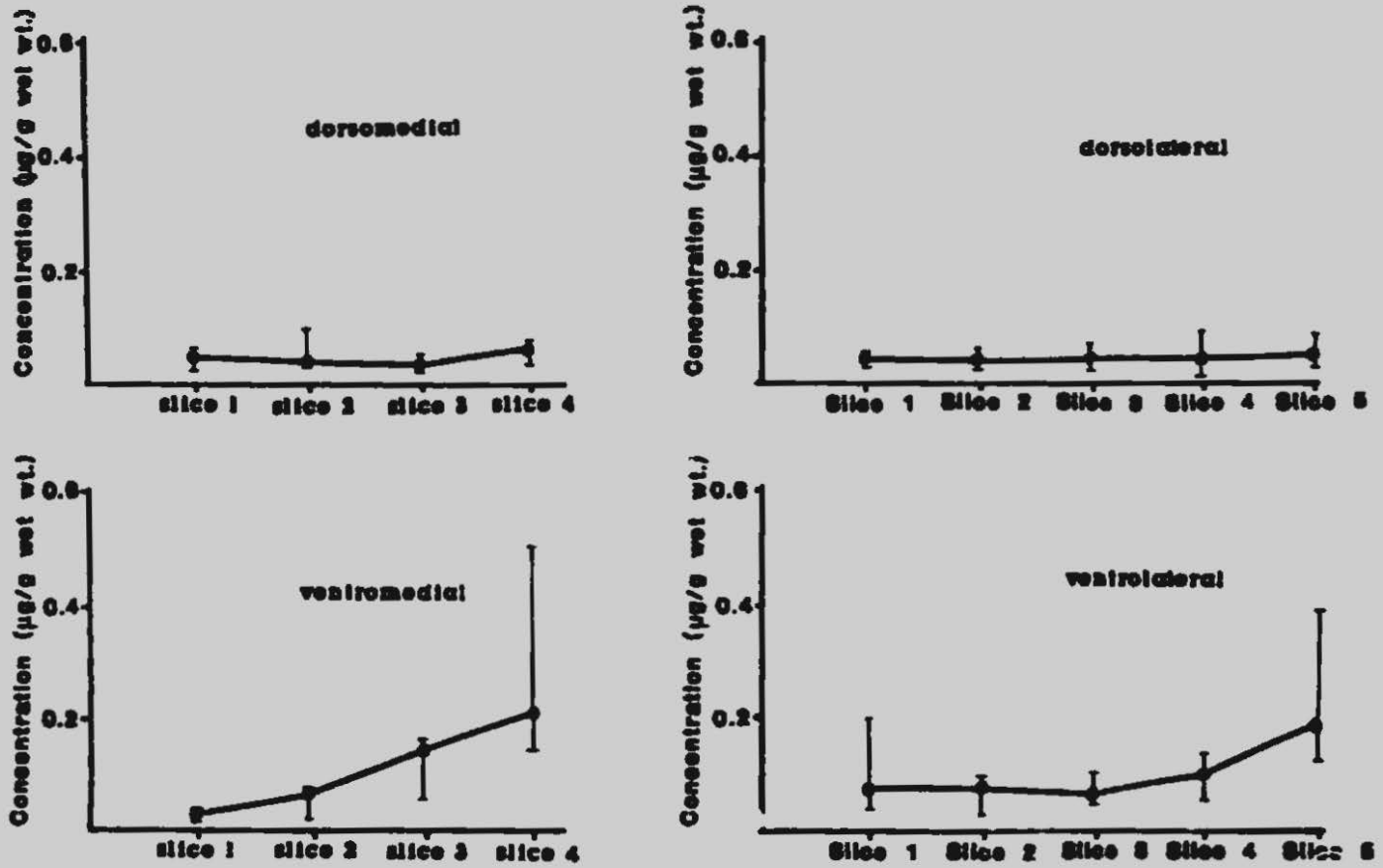

Fig. 4.10 Distribution of NA in quadrants of 5 coronal slices of the rat brain containing the striatum as depicted in Fig. 3.2. The medians of 6 observations with their ranges are shown.

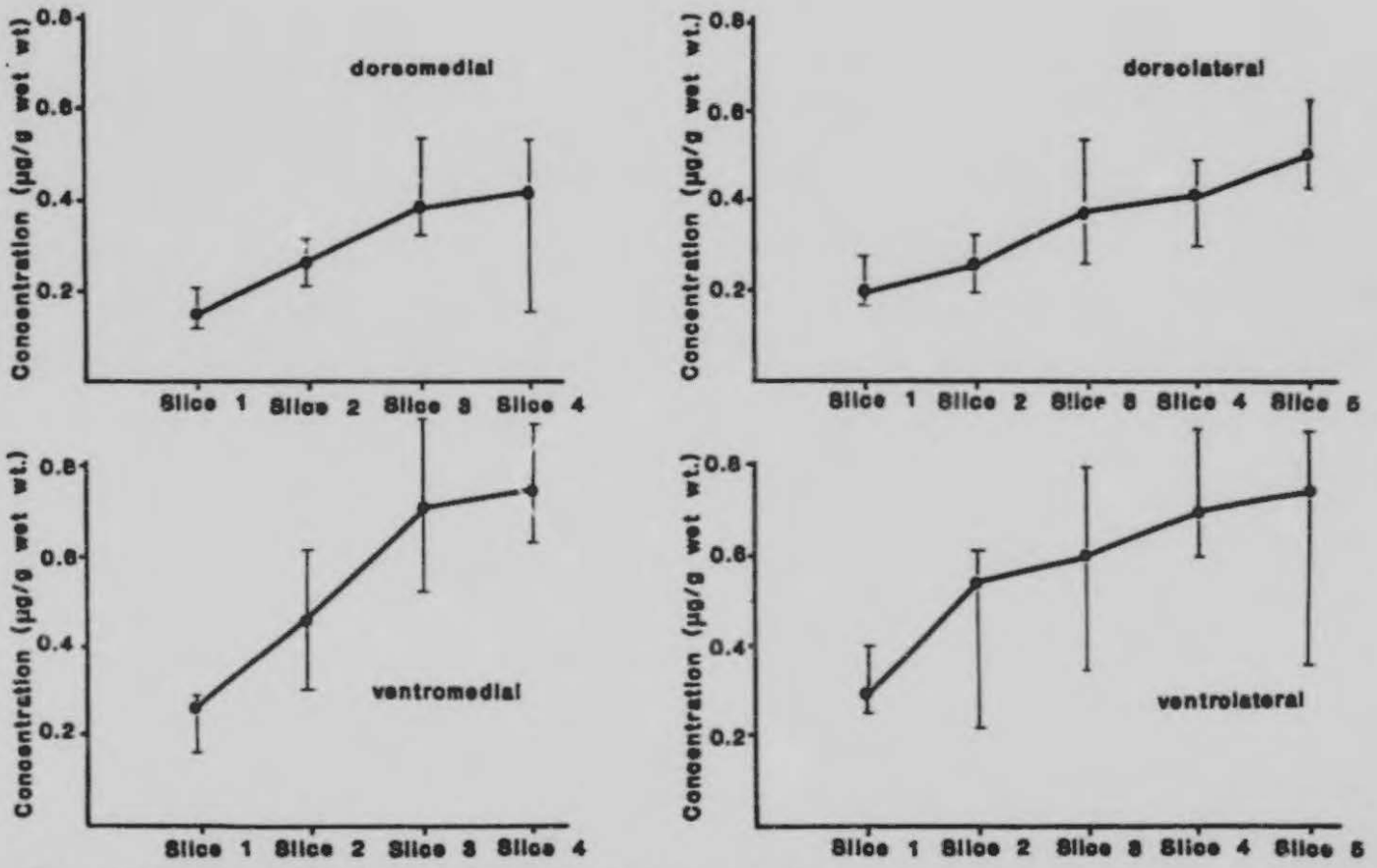

Fig. 4.11 Distribution of 5-HT in quadrants of 5 coronal slices of the rat brain containing the striatum as depicted in Fig. 3.2. The medians of 5 observations with their ranges are shown.

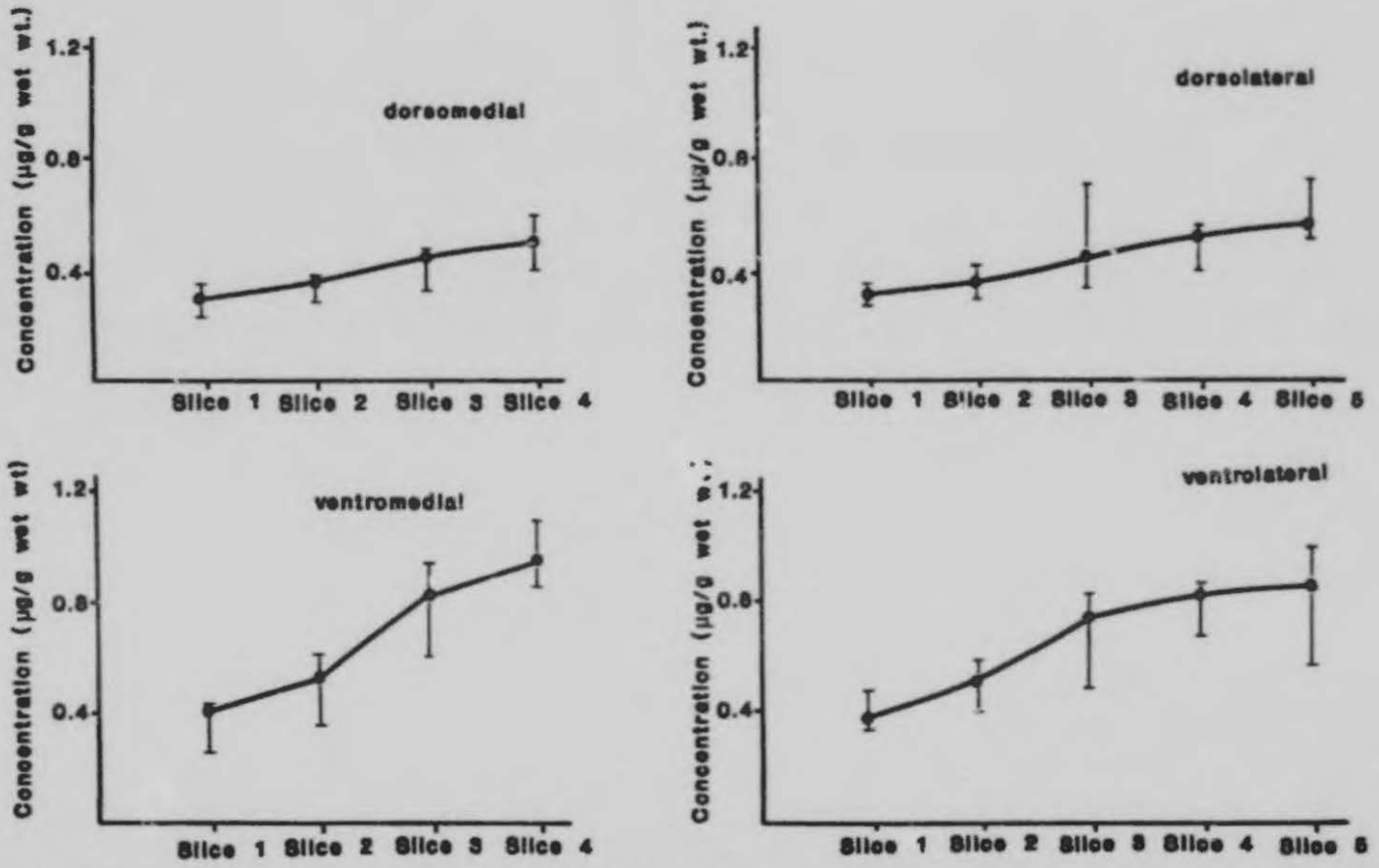

Fig. 4.12 Distribution of 5-HIAA in quadrants of 5 coronal slices of the rat brain containing the striatum as depicted in Fig. 3.2. The medians of 5 observations with their ranges are shown.

TABLE 4.4

Metabolite/ monoamine ratios in different areas of the rat striatum

Ratio	Slice	Ventromedial	Ventrolateral	Dorsomedial	Dorsolateral
DOPAC/ DA	1	0.310± 0.072	0.346± 0.046	0.236± 0.018	0.205± 0.013
	2	0.274± 0.019	0.280± 0.066	0.256± 0.046	0.207± 0.043
	3	0.317± 0.044	0.219± 0.023	0.278± 0.037	0.226± 0.033
	4	0.458± 0.045	0.249± 0.016	0.382± 0.057	0.262± 0.019
	5		0.374± 0.037		0.296± 0.016
HVA/DA	1	0.075± 0.019	0.104± 0.010	0.058± 0.005	0.073± 0.008
	2	0.089± 0.009	0.109± 0.021	0.068± 0.011	0.080± 0.010
	3	0.100± 0.012	0.100± 0.019	0.078± 0.009	0.088± 0.011
	4	0.204± 0.029	0.076± 0.020	0.111± 0.014	0.073± 0.019
	5		0.113± 0.019		0.093± 0.007
HVA/ DOPAC	1	0.251± 0.072	0.315± 0.091	0.249± 0.048	0.356± 0.047
	2	0.328± 0.073	0.404± 0.069	0.269± 0.031	0.413± 0.073
	3	0.330± 0.094	0.456± 0.133	0.293± 0.090	0.411± 0.131
	4	0.447± 0.108	0.392± 0.068	0.297± 0.063	0.357± 0.069
	5		0.295± 0.048		0.313± 0.039
5-HIAA/ 5-HT	1	1.582± 0.010	1.318± 0.055	1.839± 0.105	1.624± 0.139
	2	1.087± 0.063	1.152± 0.160	0.263± 0.076	1.501± 0.123
	3	1.094± 0.144	1.175± 0.041	1.043± 0.090	1.286± 0.037
	4	1.316± 0.119	1.080± 0.069	1.420± 0.268	1.297± 0.063
	5		1.265± 0.124		1.150± 0.100

Results are the mean± SEM of 5 observations. A two way ANOVA model was fitted to each of the 6 data sets with factors RAT and SLICE. No significant differences were observed.

DOPAC/DA ratio in the striatum was lower than that in the nucleus accumbens.

4.4 DISCUSSION

The regional distribution of the monoamines in the nucleus accumbens was similar in that lower concentrations were observed in the rostral than in the caudal area. DA concentrations were considerably higher than NA and 5-HT concentrations. In contrast to the findings of de France et al., (1983), who reported a homogeneous distribution of DA in the nucleus accumbens of the rabbit, the concentrations of DA and its metabolites DOPAC and HVA were found to be significantly higher in the medial and caudal areas than in the rostral area of the nucleus

accumbens of the rat Significant dorsoventrai differences were also observed (Fig 4 1, Table 4 1). [^{3} H]DA uptake was shown to be significantly lower in the rostral part of the nucleus accumbens than in the medial part (Allin et al., 1988, and unpublished data; Section 7 3.3) and significantly lower in the ventrorostral area than in the dorsorostral, dorsomedial or ventromedial areas (unpublished data. Section 7 3 3). The lower [^{3} H]DA uptake suggests fewer DA terminals rostrally than medially and significantly fewer ventrorostrally than dorsorostrally The apparent ratio of terminals dorsally.ventrally was 2·1, and this corresponds with the ratio of DA content which was 2 5 1 There were no dorsoventral differences found in DA uptake in the medial slice of the nucleus accumbens These findings are in contrast with those of Chronister et al., (1980) who reported a more homogeneous pattern of DA terminals in the rostral than in the caudal nucleus accumbens of the rat following an injection of horseradish peroxidase into the VTA.

In agreement with de France et al., (1983), NA concentrations were found to be significantly higher in the caudal area of the nucleus accumbens than the rostral and medial areas No dorsoventral differences were observed The NA concentration was lowest in the dorsomedial area The nucleus accumbens has previously been reported to be innervated by both LC (O'Donohue et al., 1979, Swanson and Hartman, 1975) and subcoeruleus noradrenergic neurons (Nieuwenhuys et al., 1982, Lindvall and Stenevi, 1978, O'Donohue et al., 1979), however, the precise area of termination of these two systems within the nucleus accumbens has not been established Consistent with evidence of innervation of the nucleus accumbens by the dorsal raphe (Azmitia and Segal, 1978) appreciable levels of 5-HT and its metabolite 5-HIAA were found in the nucleus accumbens The concentration of 5-HT was highest in the ventrocaudal, dorsocaudal and ventromedial areas of the nucleus accumbens and a decreasing ventrocaudal/dorsorostral concentration gradient was evident These results show that there is considerable overlap between the various monoaminergic terminals in the nucleus accumbens but that there are nevertheless finer differences in their distributions.

Metabolite/monoamine ratios were determined in order to provide an index of monoamine turnover. The DOPAC/DA and HVA/DA ratios decreased rostrocaudally. This implied that the highest DA turnover coincided with the lowest DA content in the rostral nucleus accumbens. The 5-HIAA/5-HT ratio was found to be highest in the dorsorostral area than in the ventrocaudal area The difference was not as marked as that observed with the DOPAC/DA ratio but also supports a descending rostrocaudal distribution of MAO activity. There were no differences in HVA/DOPAC ratios which suggested a uniform COMT activity throughout the nucleus accumbens.

In the striatum DA levels were found to be significantly higher rostrally than caudally, which is in agreement with Tassin et al., (1976) who reported a DA concentration of 124µg/g protein in the rostral striatum and 53µg/g protein in the caudal striatum. If 1g of tissue has a protein concentration of approximately 100mg (Gardner et al., 1987) these values would be 12400 ng/g and 5300 ng/g tissue for rostral and caudal areas respectively and would be consistent with the values obtained in this study which were 11037 ng/g tissue for the rostral striatum and 3718 ng/g tissue for the caudal striatum Beal and Martin, (1985) also reported higher DA concentrations in the rostral striatum (37-39 nmol/mg protein) than in the caudal area (17-28 nmol/mg protein). In support of these authors (Tassin et al., 1976, Beal and Martin, 1985), no dorsoventral differences in DA concentration in the striatum were shown in this study As with DA concentration in the striatum, [^{3}H]DA uptake has also been shown to be significantly greater in the rostral than in the caudal striatum (Doucet et al., 1986; Tassin et al., 1976) and this suggests a greater number of dopaminergic terminals in the rostral than the caudal area. The globus pallidus was found to contain lower DA concentrations than the striatum, which is in agreement with previous reports of DA levels in the globus pallidus (Beal and Martin, 1985, Hardy et al., 1987, Versteeg et al., 1976). Overlap of the globus pallidus into the ventromedial part of slice 3 may account for this area having significantly lower DA than any other area of the same slice. [^{3}H]DA uptake has been reported to be lower in the globus pallidus than the striatum (Hardy et al., 1987), and Lindvall and Björklund, (1978) have also reported sparse

dopaminergic innervation of this area. It has been suggested that the DA present in the globus pallidus is derived from axons passing through to the striatum rather than from terminals in this area (Versteeg et al., 1976). In the present study DA levels were found to be highest in the two most rostral slices of the striatum The nucleus accumbens in these two slices also had the highest DA levels. It is possible therefore that dopaminergic terminals from the substantia nigra and VTA of the midbrain to the striatum and nucleus accumbens is concentrated in this plane.

In this study the presence of NA in the striatum was demonstrated. NA concentrations ranged from 30 to 127 ng/g tissue in areas excluding the globus pallidus NA levels were 19.3 to 369 1 times lower than DA concentrations and more homogeneously distributed than in the nucleus accumbens. NA levels were similar in the dorsomedial and dorsolateral areas throughout the striatum, and NA levels in the ventrolateral quadrant were also similar throughout the striatum NA levels in the ventromedial areas were observed to increase along the rostrocaudal gradient There has been conflicting evidence concerning noradrenergic innervation of the striatum O'Donohue et al., (1979) provided evidence that the striatum is innervated by the ventral noradrenergic bundle as they observed decreases in NA concentration in the striatum after lesions of this noradrenergic pathway Lindvall and Björklund, (1974), using a fluorescence method, and Jones and Moore, (1977), in an autoradiographic study indicated that noradrenergic innervation of the striatum was from the locus coeruleus. Other reports, however, provide no evidence for the presence of NA (Versteeg et al., 1976) or noradrenergic innervation in the striatum (Doucet et al., 1986; Byrum and Guyenet, 1987). The globus pallidus has previously been shown to be the only area of the striatum where NA could be detected (Versteeg et al., 1976) and therefore the higher levels of NA in the ventromedial part of slice 4 and possibly also the ventromedial part of slice 3 may be due to the presence of the globus pallidus in these areas A NA concentration of 5 3 pg/µg protein (equivalent to 530 ng/g tissue as estimated above) was reported for the globus pallidus This is somewhat higher than the concentration found in the present study (261 ng/g tissue).

but differences could be due to interlaboratory or rat to rat variation. In the most caudal slice of the striatum the NA concentration in the ventral area was found to be higher than in the dorsal area and it would therefore appear that NA is concentrated in the ventrocaudal part of the striatum.

The distribution of adrenergic receptors does not appear to correspond to noradrenergic innervation in the rat brain. In autoradiographic studies, moderate to low levels of α_1 receptor binding using the radioligand [^{3}H]prazosin (Rainbow and Biegon, 1983) and [^{125}I]HEAT (Sargent Jones et al., 1985) were found in the caudate-putamen and nucleus accumbens [^{3}H]idazoxan binding, indicating the presence of α_2 receptors, appeared to be concentrated in the head and tail of the caudate nucleus while binding of this ligand was of only moderate intensity in the nucleus accumbens and low intensity in the globus pallidus (Boyajian et al., 1987). High densities of β_1 receptors and relatively low densities of β_2 receptors have been reported to be present in the caudate-putamen and nucleus accumbens (Lorton and Davis, 1987)

In the present study the concentrations of 5-HT and 5-HIAA were found to be significantly higher in the ventral than the dorsal striatum and to be higher in the caudal than the rostral area of the striatum. This is in agreement with other workers. Beal and Martin, (1985), reported 5-HT and 5-HIAA levels to be 2-3 times higher ventrally than dorsally and also reported 5-HT levels to increase along the rostrocaudal axis. In studies using autoradiographic measurement of [^{3}H]5-HT uptake and immunohistological analysis of 5-HT, Soghomonian et al., (1987), reported the density of uptake and 5-HT to be 2-3 times higher in the ventral than the dorsal part of the striatum and also that there was a slight rostrocaudal increase. These results provide evidence for a greater concentration of serotonergic terminals in the ventral than the dorsal striatum with slightly more being found in the caudal part of the striatum.

Regional differences in DA turnover were not observed in the striatum, as were found in the nucleus accumbens. Values of the DOPAC/DA ratio ranging between 0.21 and 0.38 obtained in

the present study compare favourably with values ranging between 0.10 and 0.15 reported by Beal and Martin, (1985). In the present study the DOPAC/DA ratio was lower in the striatum than in the nucleus accumbens. This implies lower MAO activity in the striatum than the nucleus accumbens, a finding which has previously been suggested by Walsh et al., (1981), and reported by Beal and Martin, (1985), who found a DOPAC/DA ratio of 0.58 in the nucleus accumbens. HVA/DOPAC ratios were found to be higher in the striatum than the nucleus accumbens, suggesting higher COMT activity in the striatum than the nucleus accumbens. 5-HIAA/5-HT ratios appeared to be slightly higher in the striatum than tne nucleus accumbens. Ratios between 1.1 and 1.8 agree favourably with those reported by Beal and Martin, (1985) who found ratios between 0.7 and 1.4. The 5-HIAA/5-HT ratio and therefore the turnover of 5-HT in both the nucleus accumbens and the striatum showed an inverse relationship with the 5-HT levels and this may indicate saturation of MAO by the monoamine, in areas of higher concentration. No such relationship was evident for the turnover of DA.

It is apparent therefore that the distribution of the monoamines DA, NA and 5-HT was heterogeneous in both the nucleus accumbens and striatum. However within each of these areas a significant degree of overlap between the monoamines was observed and interactions may therefore be possible.

4.5 SUMMARY

A detailed investigation of the distribution and turnover of the monoamines DA, NA and 5-HT and the metabolites DOPAC, HVA and 5-HIAA in the nucleus accumbens and striatum was performed. In the nucleus accumbens DA levels were low rostrally, being significantly lower ventrorostrally than dorsorostrally, and high medially and caudally. DOPAC was observed to follow a similar trend. NA was also observed to be concentrated in the caudal area of the nucleus accumbens. The concentration of 5-HT was found to be highest in the ventromedial, dorsocaudal and ventrocaudal areas of the nucleus accumbens, while 5-HIAA appeared to be

more evenly distributed. Turnover of DA and 5-HT was higher rostrally than caudally indicating a decreasing rostrocaudal activity of MAO. In the striatum DA levels were higher rostrally than caudally; no significant dorsoventral differences were observed The lowest levels of DA were found in the globus pallidus. The concentration of NA was low in the striatum and was more evenly distributed than in the nucleus accumbens. The levels of NA were found to be higher in the globus pallidus than in the striatum In the striatum 5-HT and 5-HIAA levels were found to be greater in the ventral than the dorsal areas and to increase along the rostrocaudal axis. Ratios of DOPAC/DA, HVA/DA and DOPAC/HVA appeared to be more evenly distributed in the striatum than the nucleus accumbens. The DOPAC/DA ratio was lower in the striatum indicating lower MAO activity in this area. The results of this study provided evidence that the distribution of the monoamines DA, NA, 5-HT and the metabolites DOPAC, HVA and 5-HIAA in the nucleus accumbens and striatum was heterogeneous and that there was considerable overlap between these neurotransmitter terminals.

CHAPTER 5

EFFECT OF NORADRENERGIC DENERVATION ON MONOAMINES IN THE NUCLEUS ACCUMBENS

5.1 INTRODUCTION

The nucleus accumbens has been reported to be innervated by noradrenergic neurons originating from both the LC via the dorsal noradrenergic bundle (O'Donohue et al., 1979; Swanson and Hartman, 1975) and from the subcoeruleus nuclei via the ventral noradrenergic bundle (Lindvall and Stenevi, 1978; O'Donohue et al., 1979; Nieuwenhuys et al., 1982, Section 1.2.2). The regional distribution of NA in the nucleus accumbens was described in Chapter 4 Concentrations were found to be highest in the caudal nucleus accumbens, suggesting that the greatest number of noradrenergic terminals in the nucleus accumbens are in this area However the exact source and distribution of the noradrenergic input to the nucleus accumbens has not been established Interactions between the noradrenergic and other neurotransmitter systems in the nucleus accumbens have been reported (O'Donohue et al., 1979; Manier et al., 1987, Pradhan and Bose, 1978). Activation of noradrenergic receptors has been shown to modulate [^{3}H]DA release from DA terminals in the nucleus accumbens of the rat brain (Nurse et al., 1984) and noradrenergic modulation of mesolimbic DA function has been suggested to be important in the mechanism of action of antidepressant drugs such as desipramine (Nurse et al., 1985). The effects of noradrenergic denervation on monoamines in the nucleus accumbens was therefore investigated. The neurotoxin DSP4, which has been shown to be selective for NA neurons arising in the LC (Jonsson et al., 1981; Lookingland et al., 1986, Section 1.2.4), was used and direct LC lesions were made by stereotaxically injecting 6-OHDA into this area In order to compare the effects of selective LC lesions with lesions of both the dorsal and ventral noradrenergic pathway, the MFB was lesioned by direct injection of 6-OHDA at the level of the caudal hypothalamus (Section 1.2.2, Fig. 3.3) so that neurons from

the subcoeruleus nuclei would also be lesioned. NA levels in the frontal cortex, hippocampus and hypothalamus were used as an indication of the extent and selectivity of the lesions

5.2 METHODS

Male Wistar rats were given intraperitoneal injections of DSP4 (50mg/kg) freshly dissolved in saline, or saline 10 days prior to being sacrificed This experiment was repeated providing a second set of data. Bilateral LC and MFB lesions were performed as described in Section 3 2.2 Rats weighing 250-280g were sacrificed by decapitation, the brains rapidly removed and placed on ice The nucleus accumbens was dissected into 3 areas as described in Section 3.2.1. For LC and MFB lesioned rats, the frontal cortex, hippocampus and hypothalamus were dissected out as described in Section 3.2 1. For DSP4 treated rats the frontal cortex and hippocampus were dissected out for the second data set only Perchloric acid extracts of tissue from individual rats were analysed by HPLC with electrochemical detection as described in Section 3 2 3

5.3 RESULTS

5.3.1 Effect of noradrenergic denervation on NA concentrations in the frontal cortex, hippocampus and hypothalamus

In a separate study in this laboratory DSP4, given 10 days previously, caused decreases in NA concentration of 87±2.8% in the frontal cortex, greater than 95% in the hippocampus and 26±3.8% in the hypothalamus (Russell et al., 1989). In the present study, DSP4 lesions resulted in decreases of 32-91% in NA concentration in the frontal cortex and decreases of 86-100% in the hippocampus (Table 5.1). LC lesions caused a 76-87% reduction in NA concentration in the frontal cortex, while the NA levels were decreased by 56-71% in the hippocampus and 16-41% in the hypothalamus. MFB lesions resulted in a 71-97% decrease in NA concentration in the frontal cortex and a 61-83% decrease in NA in the hypothalamus

TABLE 5.1

Effect of DSP4, LC and MFB lesions on NA concentration in different rat brain areas.

	Area		
Treatment	Frontal Cortex	Hippocampus (percentage lesion)	Hypothalamus
DSP4	70	86	ND
	77	100	ND
	32	93	ND
	74	93	ND
	91	100	ND
LC lesion	76	71	16
	83	65	16
	87	56	41
MFB lesion	95	93	83
	97	91	79
	74	84	79
	97	78	58
	71	100	68
	84	100	61

Values are from individual rats following single i.p injection of DSP4 (50mg/kg), or following 6-OHDA lesions of the LC and MFB. ND = not determined. Control values for frontal cortex, hippocampus and hypothalamus were 232.4, 302.9 and 1827 ng/g wet wt. respectively

(Table 5 1).

5.3.2 Effect of DSP4 lesions on monoamines in the nucleus accumbens

DSP4 treatment significantly decreased the NA concentration in the dorsorostral and ventrorostral areas of the nucleus accumbens, whereas NA in the medial and caudal areas was unaffected (Table 5.2, Fig. 5.1). The concentration of DA and its metabolites DOPAC and HVA, and 5-HT and its metabolite 5-HIAA were not affected by DSP4 treatment.

The HVA/DOPAC ratio was significantly increased by DSP4 treatment in the ventromedial and ventrocaudal nucleus accumbens (Table 5.3). Similar results were obtained in a separate experiment (Table 5.4) except that significant increases in HVA/DOPAC ratio were only observed in treated rats in the ventrocaudal area of the nucleus accumbens (Table 5 5)

TABLE 5.2

Effect of DSP4 treatment (50mg/kg i p 10 days previously) on the monoamine content of different areas of the rat nucleus accumbens Data Set I

			Concentration (ng/g wet wt.)		
Monoamine	Area	Treatment	Rostral	Medial	Caudal
NA	dorsal	control	321± 31	182± 24	639± 58
		DSP4	204± 17*	183± 19	618± 77
	ventral	control	349± 42	244± 29	759± 162
		DSP4	194± 21*	232± 11	577± 126
DA	dorsal	control	5040± 828	9292± 612	7271± 1138
		DSP4	3981± 740	8341± 622	5770± 750
	ventral	control	1841± 534	7905± 644	7504± 729
		DSP4	1640± 377	6130± 885	7141± 1088
DOPAC	dorsal	control	2873± 960	3807± 864	2425± 871
		DSP4	1923± 671	3080± 581	1948± 797
	ventral	control	1502± 556	3375± 669	3143± 1175
		DSP4	1028± 366	2251± 610	1964± 742
HVA	dorsal	control	530± 113	801± 201	529± 194
		DSP4	428± 127	657± 81	456± 156
	ventral	control	369± 128	739± 133	643± 265
		DSP4	294± 69	605± 100	530± 139
5-HT	dorsal	control	460± 145	495± 76	707± 163
		DSP4	374± 63	441± 86	732± 100
	ventral	control	634± 256	913± 157	1021± 168
		DSP4	456± 126	726± 152	832± 319
5-HIAA	dorsal	control	598± 94	643± 87	615± 140
		DSP4	462± 73	514± 71	571± 105
	ventral	control	715± 128	726± 64	818± 101
		DSP4	469± 94	573± 103	651± 127

Results are the mean± SEM of 5-7 observations To analyse for treatment differences the Mann-Whitney non parametric test was used *Significantly different from control, $P < 0.01$. This experiment was repeated providing a second set of data, shown in Table 5.4.

5.3.3 Effect of LC lesions on monoamines in the nucleus accumbens

LC lesions significantly decreased the NA concentration in the dorsorostral and ventrorostral areas of the nucleus accumbens (Table 5.6, Fig 5 2). The concentration of DA, DOPAC, HVA, 5-HT and 5-HIAA were not affected by LC lesions in any area of the nucleus accumbens

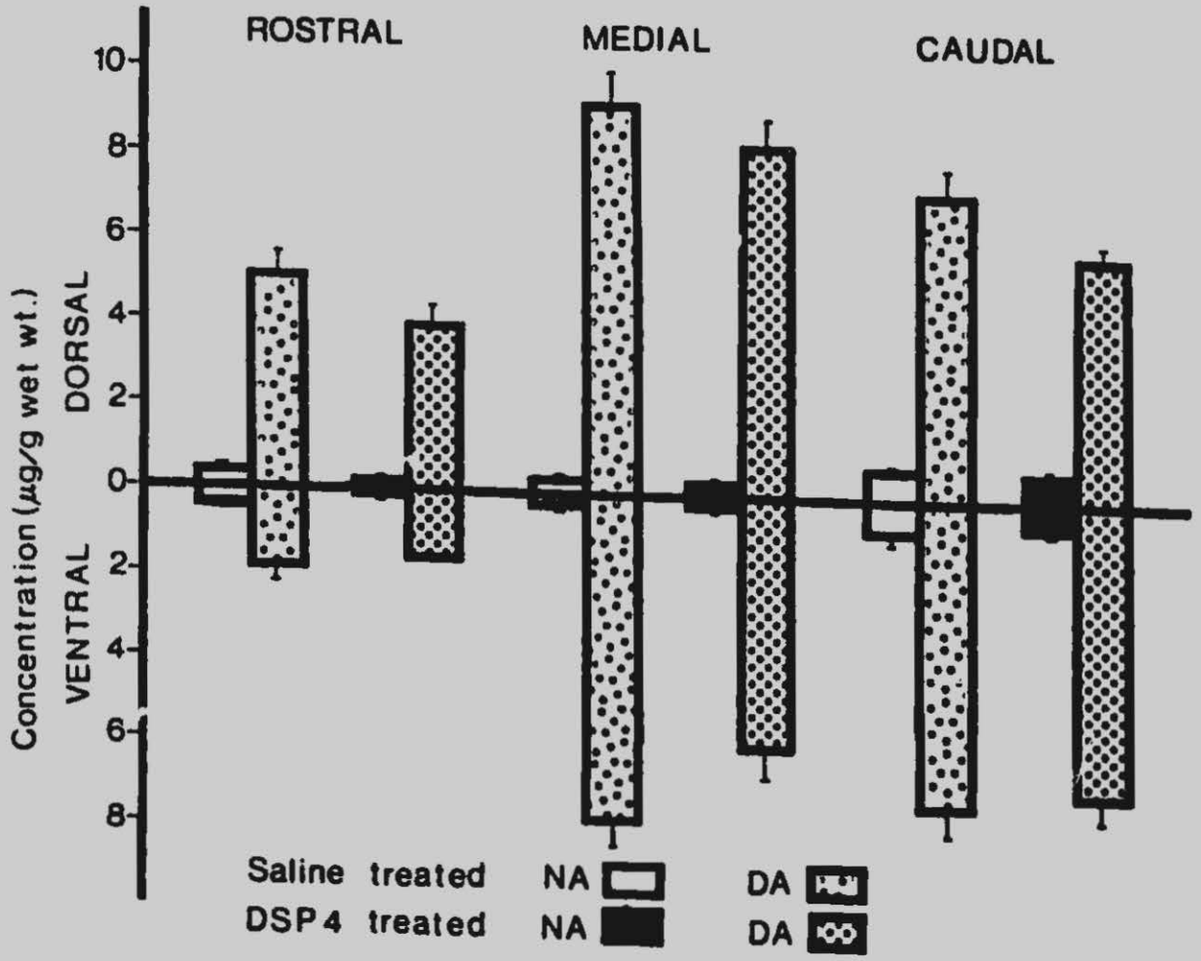

Fig. 5.1 Effect of DSP4 treatment (50mg/kg i.p. 10 days previously) on NA and DA concentrations in the nucleus accumbens of the rat. The mean ± SEM of 6-7 observations (Data Set I) are shown.

TABLE 5.3

Effect of DSP4 treatment (50mg/kg i.p. 10 days previously) on metabolite/monoamine ratios in different areas of the rat nucleus accumbens. Data Set I.

Metabolite/ monoamine ratio	Area	Treatment	Rostral	Medial	Caudal
DOPAC/ DA	dorsal	control	0.672± 0.209	0.424± 0.070	0.340± 0.041
		DSP4	0.548± 0.061	0.385± 0.041	0.345± 0.045
	ventral	control	0.946± 0.190	0.447± 0.071	0.412± 0.053
		DSP4	0.908± 0.197	0.401± 0.048	0.312± 0.060
HVA/ DA	dorsal	control	0.122± 0.032	0.089± 0.015	0.073± 0.033
		DSP4	0.114± 0.010	0.080± 0.006	0.082± 0.006
	ventral	control	0.237± 0.051	0.097± 0.013	0.085± 0.013
		DSP4	0.236± 0.056	0.097± 0.008	0.085± 0.010
HVA/ DOPAC	dorsal	control	0.194± 0.020	0.210± 0.012	0.220± 0.016
		DSP4	0.232± 0.017	0.216± 0.009	0.239± 0.012
	ventral	control	0.251± 0.016	0.221± 0.014	0.208± 0.016
		DSP4	0.311± 0.030	0.277± 0.013[a]	0.283± 0.024[b]
5-HIAA/ 5-HT	dorsal	control	1.493± 0.372	1.338± 0.167	0.884± 0.075
		DSP4	1.246± 0.049	1.199± 0.088	0.785± 0.053
	ventral	control	1.248± 0.225	0.826± 0.106	0.829± 0.107
		DSP4	1.107± 0.141	0.809± 0.056	0.860± 0.093

The results are the mean± SEM of 5-7 observations. To analyse for treatment differences the Mann-Whitney non parametric test was used. Significantly different from control, [a] P = 0.030; [b] P = 0.048. This experiment was repeated providing a second set of data, shown in Table 5.5.

The metabolite/monoamine ratios DOPAC/DA, HVA/DA HVA/DOPAC, 5-HIAA/5-HT were unchanged by lesions of the LC except in the dorsomedial area where the HVA/DOPAC ratio was found to be significantly lower in the lesioned rats than the controls (p = 0.048), (Table 5.7).

5.3 Effect of MFB lesions on monoamines in the nucleus accumbens

Lesions of the MFB decreased the NA and DA concentration in all areas of the nucleus accumbens. Significant differences were observed in all areas except in the dorsorostral area where p = 0.0547 for NA (Table 5.8, Fig. 5.3). HVA levels were significantly lower in lesioned rats than in controls in all areas of the nucleus accumbens except the dorsocaudal area

TABLE 5.4

Effect of DSP4 treatment (50mg/kg i.p. 10 days previously) on the monoamine content of different areas of the rat nucleus accumbens Data Set II.

			Concentration (ng/g wet wt.)		
Monoamine	Area	Treatment	Rostral	Medial	Caudal
NA	dorsal	control	258± 20	262± 32	836± 105
		DSP4	150± 14*	167± 27	715± 45
	ventral	control	250± 15	316± 109	1132± 156
		DSP4	145± 25*	209± 18	1034± 133
DA	dorsal	control	3661± 305	8289± 758	4274± 500
		DSP4	4741± 695	6936± 481	4276± 402
	ventral	control	1458± 333	6113± 615	5402± 507
		DSP4	2065± 381	6168± 682	5092± 268
DOPAC	dorsal	control	2073± 208	3158± 307	1488± 120
		DSP4	2343± 168	2587± 364	1167± 141
	ventral	control	1055± 131	2238± 319	1619± 221
		DSP4	1201± 60	2082± 200	1220± 201
HVA	dorsal	control	363± 23	617± 96	289± 22
		DSP4	438± 29	582± 26	335± 37
	ventral	control	268± 28	476± 51	345± 51
		DSP4	313± 21	532± 46	347± 40
5 HT	dorsal	control	362± 29	507± 69	711± 68
		DSP4	301± 21	382± 44	618± 34
	ventral	control	452± 31	760± 60	920± 37
		DSP4	427± 37	698± 62	857± 36
5-HIAA	dorsal	control	494± 30	575± 119	546± 62
		DSP4	615± 137	512± 23	703± 195
	ventral	control	558± 21	567± 41	704± 60
		DSP4	486± 24	570± 30	677± 37

Results are the mean± SEM of 5 observations To analyse for treatment differences the Mann-Whitney non parametric test was used.
*Significantly different from control, $P < 0.05$

where p = 0 0547 DOPAC levels were not as markedly decreased by MFB lesions as DA and HVA levels and were found to be significantly lower in lesioned rats in the ventrorostral area of the nucleus accumbens only. MFB lesions significantly decreased 5-HT levels in the ventrocaudal nucleus accumbens and caused significant increases in 5-HIAA concentrations in

TABLE 5.5

Effect of DSP4 treatment (50mg/kg i.p 10 days previously) on metabolite/monoamine ratios in different areas of the rat nucleus accumbens. Data Set II.

Metabolite/ monoamine ratio	Area	Treatment	Rostral	Medial	Caudal
DOPAC/ DA	dorsal	control	0 574± 0 058	0 383± 0.026	0.359± 0.035
		DSP4	0 551± 0.111	0 391± 0 081	0.270± 0.009
	ventral	control	0.862± 0 173	0.360± 0.030	0 301± 0 032
		DSP4	0.675± 0.142	0.349± 0 043	0.237± 0.032
HVA/ DA	dorsal	control	0.101± 0 009	0 074± 0 007	0.070± 0.007
		DSP4	0.125± 0 024	0.130± 0 042	0 125± 0.046
	ventral	control	0.219± 0 042	0.078± 0.005	0 063± 0.005
		DSP4	0 200± 0 032	0.126± 0 033	0.116± 0.045
HVA/ DOPAC	dorsal	control	0.180± 0 015	0 193± 0 013	0 200± 0 022
		DSP4	0.139± 0 013	0 240± 0 029	0 289± 0.009
	ventral	control	0 258± 0 014	0 220± 0 016	0 214± 0.015
		DSP4	0.262± 0 017	0.257± 0.004	0 292± 0 014*
5-HIAA/ 5-HT	dorsal	control	1.853± 0 618	1 011± 0 139	0.768± 0 099
		DSP4	2 907± 0 327	1 423± 0 202	1 118± 0.285
	ventral	control	1 220± 0 118	1 030± 0.313	0 741± 0 074
		DSP4	1 158± 0.066	0 854± 0 086	0 794± 0 052

The results are the mean± SEM of 5 observations To analyse for treatment differences the Mann Whitney non parametric test was used
*Significantly different from control, P = 0 016

the ventromedial, dorsocaudal and ventrocaudal areas of the nucleus accumbens (Table 5 8).

The DOPAC/DA ratio was significantly increased in the medial and caudal areas of the nucleus accumbens, both dorsally and ventrally, by MFB lesions (Table 5 9). The HVA/DA ratio was significantly increased in the ventrorostral, dorsomedial, ventromedial and ventrocaudal areas, and the HVA/DOPAC ratio was significantly increased in the ventrorostral area of the nucleus accumbens. MFB lesions were found to significantly increase the 5 HIAA/5-HT ratio in the medial and caudal areas of the nucleus accumbens, both dorsally and ventrally (Table 5.9).

TABLE 5.6

Effect of LC lesion on monoamines in the nucleus accumbens of the rat

			Concentration (ng/g wet wt)		
Monoamine	Area	Treatment	Rostral	Medial	Caudal
NA	dorsal	control	287± 14	170± 16	465± 244
		LC lesion	155± 14*	112± 19	327± 59
	ventral	control	260± 25	222± 52	491± 81
		LC lesion	120± 10*	145± 24	436± 127
DA	dorsal	control	1439± 511	6819± 617	6395± 986
		LC lesion	691± 425	6030± 135	6115± 968
	ventral	control	311± 73	3480± 560	5089± 841
		LC lesion	344± 140	3264± 299	5424± 1407
DOPAC	dorsal	control	1180± 286	3186± 255	2414± 347
		LC lesion	673± 256	3479± 342	3147± 367
	ventral	control	643± 251	2035± 244	2202± 305
		LC lesion	423± 110	2229± 88	1840± 407
HVA	dorsal	control	275± 10	649± 49	607± 89
		LC lesion	214± 14	611± 46	640± 52
	ventral	control	198± 43	463± 51	516± 88
		LC lesion	142± 28	424± 31	405± 102
5 HT	dorsal	control	356± 34	357± 29	562± 53
		LC lesion	289± 21	362± 16	458± 15
	ventral	control	325± 46	650± 63	925± 112
		LC lesion	246± 7.3	615± 26	1021± 110
5-HIAA	dorsal	control	498± 51	446± 27	515± 54
		LC lesion	491± 55	490± 41	506± 46
	ventral	control	576± 64	543± 31	633± 61
		LC lesion	410± 69	552± 34	590± 46

Results are the mean± SEM of 3 observations To analyse for treatment differences the Mann-Whitney non parametric test was used

*Significantly different from control, P = 0 024.

5.4 DISCUSSION

From this study and from data obtained previously (Russell et al , 1989), DSP4, given ten days prior to sacrifice, was observed to cause a significant decrease in NA concentration in the frontal cortex and hippocampus of the rat The NA concentration in the hypothalamus was observed to be less affected by DSP4 lesions (Russell et al , 1989) 6-OHDA lesions of the LC

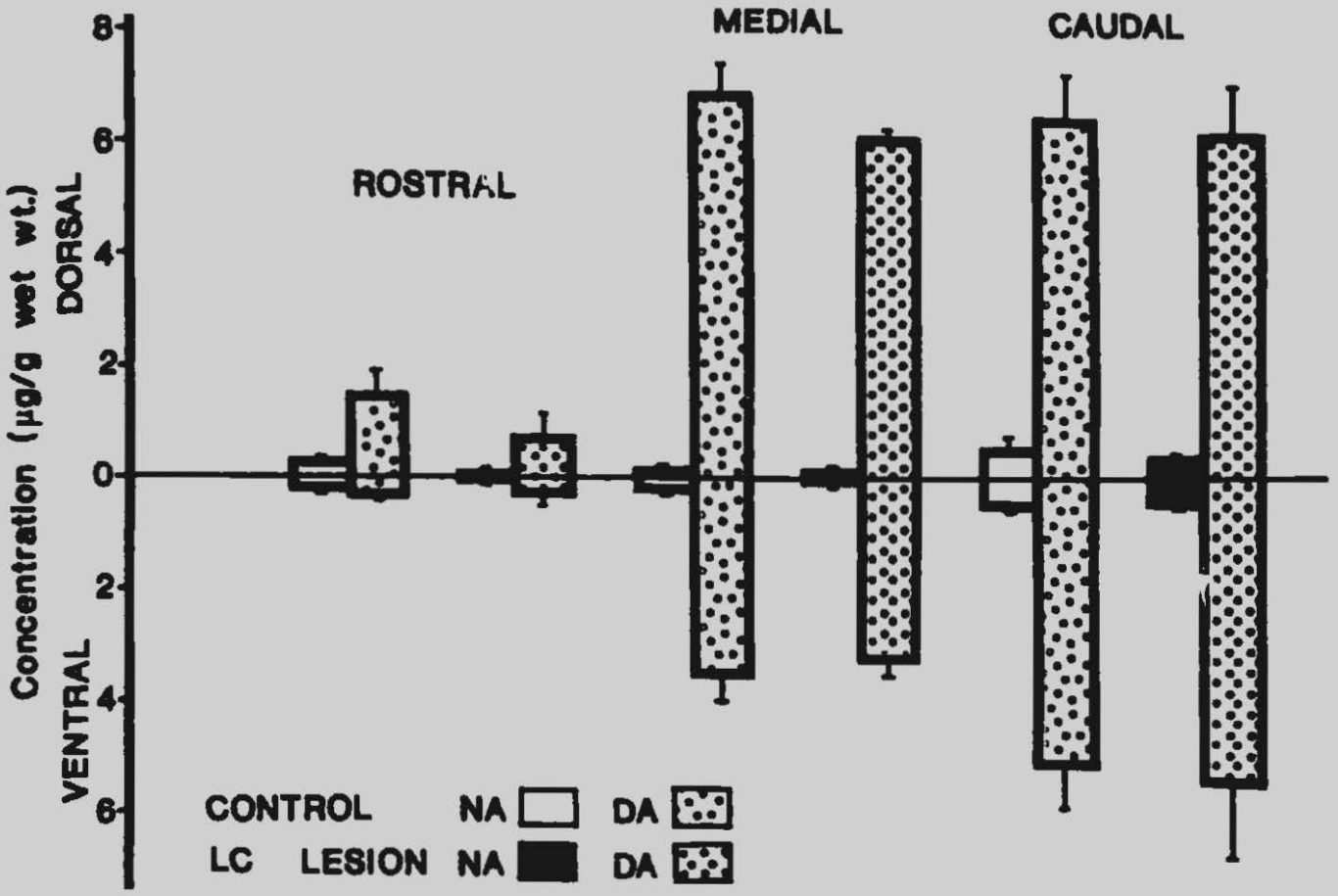

Fig. 5.2 Effect of LC lesion on NA and DA concentrations in the nucleus accumbens of the rat. The mean ± SEM of 8 observations are shown.

TABLE 5.7

Effect of LC lesion on metabolite/monoamine ratios in different areas of the rat nucleus accumbens.

Metabolite/ monoamine ratio	Area	Treatment	Rostral	Medial	Caudal
DOPAC DA	dorsal	control	0 955± 0 209	0.481± 0.052	0.399± 0.
		LC lesion	1 315± 0.278	0 575± 0.044	0.528± 0
	ventral	control	1.248± 0.215	0.610± 0.048	0 449± 0.0
		LC lesion	1 542± 0 387	0.694± 0.070	0.350± 0.022
HVA/ DA	dorsal	control	0 355± 0.011	0.097± 0.008	0.097± 0.005
		LC lesion	0 601± 0.267	0.101± 0 006	0.108± 0.011
	ventral	control	0 641± 0 248	0.141± 0 015	0.099± 0.009
DA		LC lesion	0.625± 0.260	0.133± 0.018	0.077± 0.009
HVA/ DOPAC	dorsal	control	0 528± 0 270	0 205± 0 005	0.249± 0.013
		LC lesion	0 413± 0 138	0.177± 0 010*	0.207± 0.022
	ventral	control	0.443± 0 092	0 230± 0.009	0.224± 0.020
		LC lesion	0 370± 0 074	0.192± 0.021	0 221± 0.025
5-HIAA/ 5-HT	dorsal	control	1.254± 0.248	1 100± 0 156	0 842± 0 132
		LC lesion	1.742± 0.294	1.358± 0 123	1 108± 0.117
	ventral	control	1 784± 0 489	0.747± 0 129	0 585± 0.093
		LC lesion	1 684± 0 326	0.904± 0 021	0 584± 0.038

Results are the mean± SEM of 3 observations To analyse for treatment differences the Mann-Whitney non parametric test was used

*Significantly different from control, P = 0.048

were also found to cause the greatest reductions in NA levels in the frontal cortex and hippocampus, areas known to be innervated by the LC (Lindvall and Björklund, 1978; Swanson and Hartman, 1975). In the hypothalamus, which receives substantial innervation from the subcoeruleus nuclei, NA levels were reduced by only 16-41% These results are in agreement with Logue (1985) who reported a decrease of 86% in NA in the cortex and a decrease of 91% in the hippocampus after DSP4 treatment, and with Lookingland et al., (1986) who reported greater than 80% decreases in NA levels in the frontal cortex and hippocampus, and 20-40% decreases in the hypothalamus after DSP4 lesions Also in agreement with these findings, Cole and Robbins, (1987) reported that 6-OHDA lesions of the dorsal noradrenergic bundle caused approximately 90% reductions in NA content of the cortex and hippocampus, whereas the

TABLE 5.8

Effect of MFB lesion on monoamines in the nucleus accumbens of the rat.

			Concentration (ng/g wet wt.)		
Monoamine	Area	Treatment	Rostral	Medial	Caudal
NA	dorsal	control	238± 25	159± 11	370± 41
		MFB lesion	116± 47	<54± 16[b]	130± 29[b]
	ventral	control	182± 7.5	165± 12	577± 208
		MFB lesion	<52± 14[b]	80± 15[b]	158± 35[b]
DA	dorsal	control	1121± 536	6314± 715	6884± 681
		MFB lesion	186± 85[a]	1611± 702[b]	2574± 977[a]
	ventral	control	513± 319	3575± 410	6189± 497
		MFB lesion	62± 20[a]	1188± 483[a]	2248± 508[b]
DOPAC	dorsal	control	757± 279	2066± 444	2545± 324
		MFB lesion	258± 143	1313± 459	1853± 539
	ventral	control	438± 145	1950± 106	2266± 184
		MFB lesion	83± 36[a]	933± 321	1438± 384
HVA	dorsal	control	252± 55	630± 54	596± 59
		MFB lesion	<77± 3.7[b]	275± 75[b]	364± 81
	ventral	control	167± 32	433± 31	508± 47
		MFB lesion	<84± 5 3[a]	202± 51[a]	278± 56[a]
5-HT	dorsal	control	344± 15	357± 19	572± 36
		MFB lesion	266± 27	301± 44	426± 55
	ventral	control	331± 36	668± 51	974± 37
		MFB lesion	255± 24	502± 68	694± 71[a]
5-HIAA	dorsal	control	449± 22	457± 29	452± 39
		MFB lesion	472± 24	513± 41	617± 27
	ventral	control	414± 45	515± 26	564± 36
		MFB lesion	486± 22	694± 30[b]	717± 37[a]

Results are the mean ± SEM of 6 observations To analyse for treatment differences the Mann-Whitney non parametric test was used.
Significantly different from control, [a] $P<0.05$; [b] $P<0.01$

hypothalamic NA concentration was reduced to 50% of control values. The extent of depletion of NA in the frontal cortex and hippocampus compared with the hypothalamus therefore provides evidence that LC neurons have been lesioned in preference to neurons from the subcoeruleus nuclei of the pons medulla

MFB lesions resulted in marked reductions in NA concentration in the frontal cortex

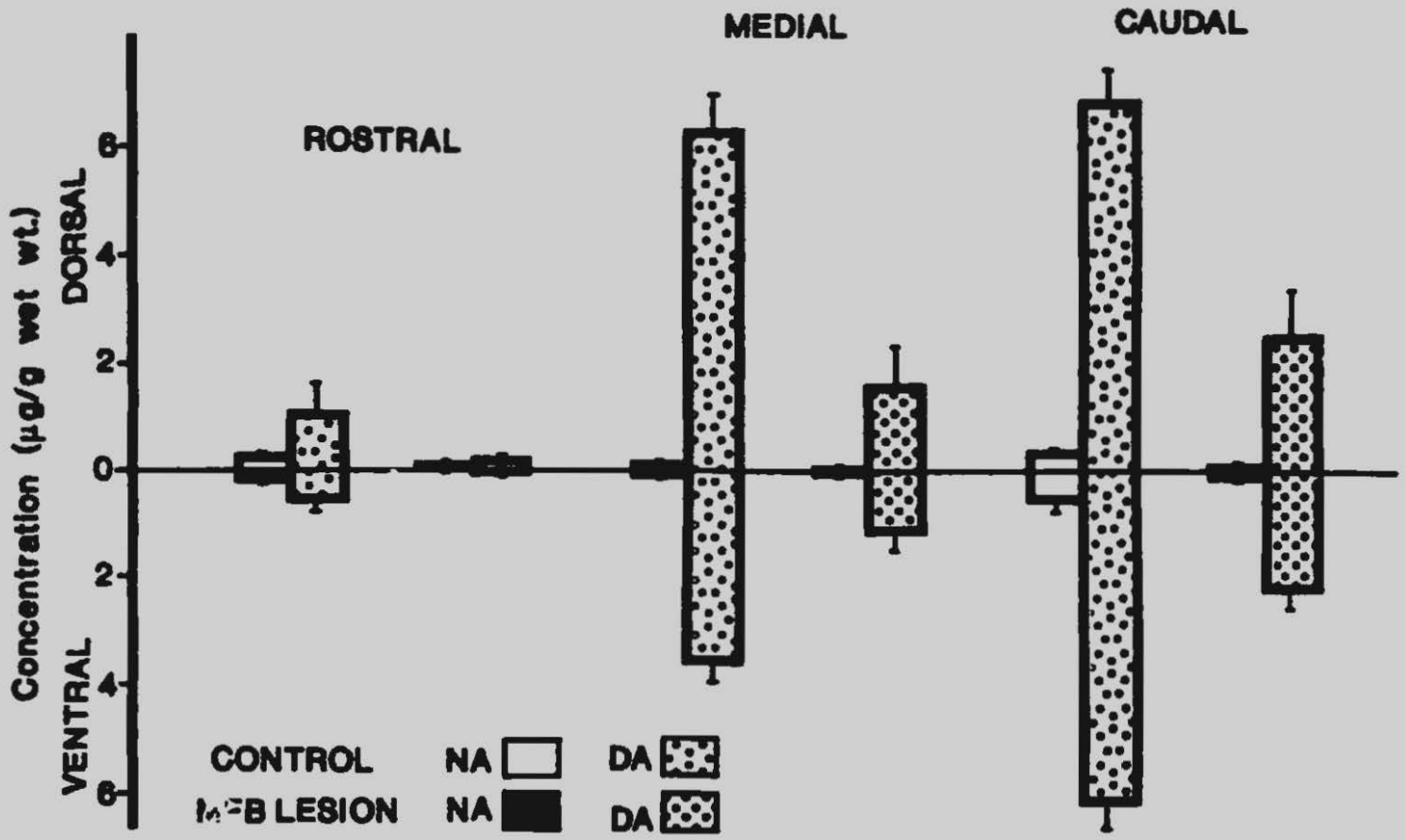

Fig. 6.8 Effect of MFB lesion on NA and DA concentrations in the nucleus accumbens of the rat. The mean ± SEM of 8 observations are shown.

TABLE 5.9

Effect of MFB lesion on metabolite/monoamine ratios in different areas of the rat nucleus accumbens.

Metabolite/ monoamine ratio	Area	Treatment	Rostral	Medial	Caudal
DOPAC/ DA	dorsal	control	0.867± 0 135	0.317± 0 064	0.367± 0.029
		MFB lesion	1.233± 0.124	1.005± 0 224*	0.887± 0.230*
	ventral	control	1 494± 0.025	0.576± 0 060	0.370± 0.028
		MFB lesion	1.238± 0.125	0.830± 0.059*	0.684± 0.164*
HVA/ DA	dorsal	control	0.419± 0.249	0.103± 0.018	0.088± 0.016
		MFB lesion	1.107± 0.424	0.396± 0.135*	0.184± 0.075
	ventral	control	0.727± 0.394	0.125± 0.021	0.083± 0.012
		MFB lesion	1.838± 0.341*	0 228± 0 037*	0.139± 0.024*
HVA/ DOPAC	dorsal	control	0 477± 0.106	0 460± 0.157	0.243± 0.015
		MFB lesion	0.964± 0 385	0.417± 0.150	0 227± 0.025
	ventral	control	0 451± 0.043	0 225± 0 019	0.226± 0 013
		MFB lesion	1.589± 0 322*	0.279± 0 053	0 217± 0.022
5-HIAA 5-HT	dorsal	control	1.336± 0.127	1 282± 0.035	0.798± 0.064
		MFB lesion	1 880± 0.231	1.969± 0.474*	1.563± 0.197*
	ventral	control	1 369± 0 024	0.785± 0.036	0 587± 0.053
		MFB lesion	1 959± 0.117	1.505± 0 191*	1 094± 0.137*

Results are the mean ± SEM of 6 observations. To analyse for treatment differences the Mann-Whitney non parametric test was used.
Significantly different from control, * $P<0.05$

and hypothalamus, providing evidence that neurons arising in the locus coeruleus and subcoeruleus nuclei of the pons medulla had been lesioned

DSP4 lesions were found to significantly decrease the NA concentration in the rostral nucleus accumbens, both dorsally and ventrally (Table 5 2, Fig. 5 1). These results were confirmed in a separate experiment (Table 5.4). 6-OHDA lesions of the LC were also found to significantly decrease the NA concentration in the rostral nucleus accumbens, both dorsally and ventrally (Table 5.6). As similar effects on the NA content in the nucleus accumbens were obtained after DSP4 and direct LC lesions, these results therefore support previous reports that the neurotoxin DSP4 is selective for neurons arising in the LC (Jonsson et al., 1981, Lookingland et

al., 1986). The results also confirm reports of LC innervation of the nucleus accumbens (O'Donohue et al., 1979; Swanson and Hartman, 1975; Russell et al., 1989), and in addition provide evidence that noradrenergic terminals from the LC are confined to the rostral nucleus accumbens.

Concentrations of DA in the nucleus accumbens were not affected by DSP4 or LC lesions which is in agreement with several previous reports (Jonsson et al., 1981 Lookingland et al., 1986, Logue, 1985). In contrast, several studies have shown DA levels in the cerebral cortex (Harik, 1984; Berger 1988) or DA mediated behaviours (Donaldson et al., 1976) to be increased after DSP4 or 6-OHDA lesions of the LC.

The turnover of DOPAC was found to be significantly higher in the ventrocaudal (Table 5 3) and ventromedial and ventrocaudal (Table 5 5) areas of the nucleus accumbens after DSP4 treatment, demonstrated by an increase in HVA/DOPAC ratio These findings suggest an increase in COMT activity in these areas of the nucleus accumbens following noradrenergic denervation and a shift to extraneuronal metabolism of DOPAC by COMT The increased DOPAC turnover may be more evident in the medial and caudal areas of the nucleus accumbens because of the greater number of DA terminals located in these areas (Allin et al., 1988) In contrast to these findings Harik, (1984) reported increases in DA, DOPAC and HVA levels in the cerebral cortex after 6-OHDA lesions of LC neurons The results of Harik, (1984) were suggested to be due to proliferation of dopaminergic terminals to compensate for degenerated noradrenergic terminals following chronic NA denervation However, greater reductions in NA were observed in the cerebral cortex than in the nucleus accumbens after DSP4 lesions and this may explain the lack of effect of DSP4 treatment on DA, DOPAC and HVA levels in the present study Similar changes in DA turnover were not observed in LC lesioned rat nucleus accumbens, in contrast to the report of Harik, (1984) An explanation for the increased DA turnover after DSP4 treatment and lack of effect on the DA neurons after LC lesion could perhaps be due to the different mechanisms by which these neurotoxins act

(Jonsson et al., 1981)

5-HT levels and turnover in the nucleus accumbens were not affected by DSP4 or 6-OHDA lesion of the LC, which is in agreement with previous reports (Lookingland et al., 1986; Harik, 1984) and demonstrates the selectivity of the lesions. However, Logue, (1985) reported a significant decrease in 5-HT levels in the striatum after DSP4 treatment

MFB lesions were found to cause a considerably more widespread decrease in NA levels in the nucleus accumbens (Table 5.8, Fig. 5.3). These findings support those of Lindvall and Stenevi, (1978) who reported that noradrenergic neurons of the ventral noradrenergic bundle passed through the medial part of the nucleus accumbens O'Donohue et al., (1979) have also reported a significant reduction in NA concentration in the nucleus accumbens after lesions of the ventral bundle NA neurons, and Plaznik et al., (1982) reported similar decreases in NA levels in the forebrain of rats after ventral bundle lesions In the present study the concentration of NA was shown to be significantly reduced in all areas of the nucleus accumbens except in the dorsorostral area where the decrease was slightly less marked ($p = 0.0547$) The lesions were placed ventrally in the MFB (Fig 3 3) and it is therefore possible that neurons from the dorsal noradrenergic bundle which are located dorsally in the MFB were not affected by the 6-OHDA lesion to the same extent as the ventrally situated neurons arising from subcoeruleus nuclei This would imply that there is an overlap in the rostral areas, the rostral nucleus accumbens is innervated by LC neurons as well as subcoeruleus neurons whilst medial and caudal areas of the nucleus accumbens receive their innervation from the subcoeruleus neurons only

The concentrations of DA and HVA were significantly decreased in all areas of the nucleus accumbens after 6-OHDA lesions of the MFB. DOPAC levels were found to be significantly decreased in the ventrorostral area of the nucleus accumbens. These results are in support of reports that DA neurons from the substantia nigra and VTA form part of the MFB (Moore and Kelly, 1978, Oades and Halliday, 1987, Lindvall and Stenevi, 1978) and that some of the MFB DA neurons pass through the nucleus accumbens on their way to the lateral septal nucleus

(Lindvall and Stenevi, 1978) DA neurons in the MFB have been reported to have several branches One has been shown to pass dorsally from the MFB into the nucleus accumbens and another has been observed to continue further rostrally and enter the nucleus accumbens at a more anterior level (Lindvall and Björklund, 1974; Lindvall and Björklund, 1978)

The DOPAC/DA and HVA/DA ratios were significantly increased in the medial and caudal parts of the nucleus accumbens, while the HVA/DA and HVA/DOPAC ratios were increased in the ventrorostral area of the nucleus accumbens after MFB lesions This implies an increase in MAO activity in the medial and caudal areas which are innervated by subcoeruleus NA neurons only while COMT activity appears to be increased in the ventrorostral nucleus accumbens after MFB lesioning These changes could be due to different compensatory increases in the activity of the dopaminergic system resulting from the different influence of LC and subcoeruleus NA neurons DA concentrations were observed to be significantly higher in the medial and caudal areas of the nucleus accumbens than in the rostral area (Allin et al., 1988), and after MFB lesioning a greater number of DA neurons could remain intact in these areas. This would allow more intraneuronal formation of DOPAC by MAO than in the rostral areas, and in support of this suggestion the DOPAC concentration was found to be significantly lower in the ventrorostral area than in any other area of the nucleus accumbens (Table 5 8) In agreement with these findings, DA turnover has previously been reported to be increased in the striatum after lesions of the nigrostriatal pathway (Altar et al., 1987) It was suggested by these authors that injury of DA terminals results in loss of DA uptake sites and shifts the route of inactivation from reuptake and subsequent metabolism to DOPAC to extraneuronal conversion by COMT This hypothesis may explain the significant decrease in DOPAC levels and corresponding increase in HVA/DOPAC ratio in the ventrorostral area of the nucleus accumbens. The DA content and number of DA terminals in the ventrorostral area of the nucleus accumbens (Allin et al., 1988, and Chapters 4 and 6) was found to be significantly lower than in any other area of the nucleus accumbens After MFB lesions the DA level was decreased to almost undetectable levels (Table 5 8) suggesting almost complete dopaminergic

denervation. Metabolism of DA may therefore occur mainly extraneuronally by the action of COMT resulting in the increased HVA/DOPAC ratio observed. Robinson and Whishaw, (1988) and Stachowiak et al., (1987) have also provided evidence that after 6-OHDA or electrolytic lesions of the nigrostriatal pathway compensatory changes occur in the striatum. DOPAC/DA and HVA/DA ratios were reported to be increased so that extracellular DA concentrations were normalized, even after an apparent 99% depletion of DA in postmortem tissue. 6-OHDA lesions of the VTA were also reported to significantly decrease DA, DOPAC and HVA in the nucleus accumbens, and apparent increases in DOPAC/DA and HVA/DA ratios were calculated while no change was observed in the HVA/DOPAC ratio (Ornstein et al., 1987).

The increase in DA metabolism may also be a result of noradrenergic denervation (Harik, 1984), with compensatory increases in intraneuronal formation of DOPAC being more evident in the medial and caudal area of the nucleus accumbens where noradrenergic and dopaminergic innervation is greatest. In behavioural studies the noradrenergic system has been suggested to have an inhibitory effect on DA neuron activity (Plaznik et al., 1982) and that facilitation of DA dependent behaviours occurs when the noradrenergic system is depressed (Pradhan et al., 1978). In contrast to these findings, however, decreases in DA turnover in the nucleus accumbens after ventral noradrenergic bundle lesion have also been reported (O'Donohue et al., 1979).

MFB lesions resulted in a significant decrease in 5-HT levels in the ventrocaudal area of the nucleus accumbens, which corresponded to the area of highest 5-HT concentration (Chapter 4). This decrease may indicate loss of selectivity of 6-OHDA for catecholaminergic neurons in the concentration used (a total dose of 10µg as 10µg/µl solution), (Breese and Cooper, 1977) or it may reflect changes secondary to the NA and DA lesions. 5-HIAA levels were significantly increased in the ventromedial, dorsocaudal and ventrocaudal areas of the nucleus accumbens. These findings are in contrast to those of Plaznik et al., (1982) who observed no change in DA, 5-HT or 5-HIAA levels in the forebrain of the rat after electrolytic lesions of the ventral

noradrenergic bundle. However, these lesions were made at a more caudal level in the brain than the MFB lesion in this study and the dopaminergic cells of origin were excluded from the lesion.

The 5-HIAA/5-HT ratio was observed to be increased in the medial and caudal areas of the nucleus accumbens, as was found with the DOPAC/DA ratio after MFB lesions. This agrees with the suggestion that MAO activity was increased in these areas of the nucleus accumbens after MFB lesions. The increased activity of serotonergic neurons after MFB lesions could be a compensatory mechanism following depletion of 5-HT, however 5-HT was significantly decreased only in the ventrocaudal area of the nucleus accumbens whilst the increase in turnover was observed in both medial and caudal areas. The increased 5-HT turnover could also be due to dopaminergic denervation. Ornstein et al., (1987) reported that 5-HT was decreased and 5-HIAA increased in the nucleus accumbens following 6-OHDA lesions of the VTA. However, no change in 5-HIAA concentration was observed in the striatum following nigrostriatal lesions (Robinson and Whishaw 1988). It has been widely reported that noradrenergic denervation results in increased 5-HT turnover (Kostowski, 1980, Pradhan and Bose, 1978) and Plaznik et al., (1983) have proposed that NA released from noradrenergic terminals in the median raphe inhibits the activity of 5-HT neurons. Therefore the increased 5-HT turnover in the nucleus accumbens observed after MFB lesions in this study may also be a result of noradrenergic denervation.

5.5 SUMMARY

DSP4 treatment (50mg/kg i.p. 10 days previously) and direct LC lesion by local infusion of 6-OHDA resulted in significant decreases of NA content in the rostral nucleus accumbens, both dorsally and ventrally. The medial and caudal areas were not affected. The LC neurons therefore appear to project to the rostral part of the nucleus accumbens. DA and 5-HT concentrations were not affected by DSP4 treatment or LC lesions. However, DSP4 lesions

caused increases in HVA/DOPAC ratios in the ventromedial and ventrocaudal areas of the nucleus accumbens, suggesting a greater COMT activity in these areas. 6-OHDA lesions of the MFB resulted in depletion of NA in all areas of the nucleus accumbens, demonstrating a more widespread innervation of the nucleus accumbens by neurons of the subcoeruleus nuclei of the pons medulla. DA and HVA concentrations were also significantly decreased in all areas of the nucleus accumbens following MFB lesions, and DOPAC levels were significantly reduced in the ventrorostral area. These results are in support of widespread evidence that dopaminergic neurons arising in the VTA form part of the MFB, some of which terminate in the nucleus accumbens. DOPAC/DA ratios were significantly increased in the medial and caudal parts of the nucleus accumbens, and HVA/DA ratios were significantly increased in the dorsomedial, ventromedial and ventrocaudal areas of the nucleus accumbens. These results imply an increased DA turnover, and increased MAO activity in these areas after MFB lesion, and may be due to a compensatory increase in DA neuron activity following DA denervation. In addition compensatory increases in dopaminergic activity may be due to an effect of noradrenergic denervation, which also occurs in these areas of the nucleus accumbens. The HVA/DOPAC and HVA/DA ratios were significantly increased in the ventrorostral area of the nucleus accumbens implying an increased COMT activity in this area. 5-HT levels in the ventrocaudal area of the nucleus accumbens were significantly decreased and 5-HIAA levels were significantly increased in the ventromedial, dorsocaudal, and ventrocaudal areas following MFB lesions. The increased 5-HIAA/5-HT ratios in the medial and caudal areas of the nucleus accumbens indicate increased MAO activity as suggested by the DOPAC/DA ratios. This suggests either a lack of selectivity of the lesions for catecholaminergic neurons or may be due to secondary compensatory mechanisms resulting from the noradrenergic denervation.

CHAPTER 6

CHARACTERIZATION OF RECEPTOR BINDING IN THE NUCLEUS ACCUMBENS

6.1 INTRODUCTION

The radioligand binding assay has been used to study interactions between radioactively labelled ligands and receptors so that parameters such as the number (Bmax) and affinity (K_D), for various ligands, of receptors in a particular tissue can be determined (Leysen and Gommeren, 1981; Munson and Rodbard, 1980; Ferkany, 1987). For optimal binding conditions the ligand used must be physiologically active, chemically pure and should demonstrate stereoselectivity. It is also important for ligands to be selective i.e. only react with one receptor site, and have high affinity for the receptor site so that nonspecific binding can be maintained at the lowest level possible (Ferkany, 1987). It is also desirable for the radioligand to have a high specific activity in the event of a particular tissue containing a small absolute number of receptors. The nucleus accumbens is a major terminal area for dopaminergic neurons arising in the VTA of the midbrain (Oades and Halliday, 1987; Ungerstedt, 1971a; Lindvall and Stenevi, 1978; Chronister et al., 1980, Section 1.2.2), and DA receptor sites have been shown to be localized in this brain area (Savasta et al., 1986, Dubois et al., 1986, Hyttel and Arnt, 1987; Schulz et al., 1985a). DA receptors have been divided into two major subtypes, the DA D1 receptors which are positively coupled to adenylate cyclase and the DA D2 receptors which are negatively coupled or uncoupled to this enzyme (Kebabian and Calne, 1979; Stoof and Kebabian, 1981). A selective ligand for DA D1 receptors, SCH23390, has recently been introduced by Iorio et al., (1983). [^{3}H]SCH23390 has been reported to be highly selective for DA D1 receptor sites (O'Boyle and Waddington, 1987; Kilpatrick et al., 1986; Schultz et al., 1985a; Billard et al., 1984) and to have low nonspecific binding (Kilpatrick et al., 1986; Hyttel and Arnt, 1987; Billard et al., 1984). However, there have been reports indicating that the DA

D1 ligand has some affinity for 5-HT_1 (Skarsfeldt and Larsen, 1988) and 5-HT_2 receptor binding sites (Bischoff et al., 1986, Bischoff et al., 1988; McQuade et al., 1988). Various neuroleptics have been characterized and used for DA D2 receptor binding assays. [^{3}H]Spiperone has been reported to be a selective specific ligand for this purpose (Leysen and Gommeren, 1981; Kebabian and Calne, 1979; Laduron et al., 1978; Chatterjee et al., 1988). [^{3}H]Spiperone has also been reported to bind to 5-HT_2 binding sites (Leysen et al., 1978; List and Seeman, 1981; Schultz et al., 1985a). The nucleus accumbens has been observed to contain 5-HT_1 and 5-HT_2 binding sites (Pazos and Palacios, 1985; Pazos et al., 1985), and therefore measures should be taken to prevent the interference of 5-HT binding sites with the DA binding assay. Iodinated butyrophenones, which have the advantage of high specific activity, have also been used as radioligands for DA D2 receptor binding assays (Ferkany, 1987; Gundlach et al., 1984; Nakatsuka et al., 1987). A [illegible] different radioligand binding assay conditions have been employed in the determination of D1 (Kilpatrick et al., 1986; Hyttel and Arnt, 1987; Billard et al., 1984; Schulz, 1985b) and D2 receptor binding parameters (Leysen and Gommeren, 1981; Hamblin et al., 1984; Usdin et al., 1980). Before adopting assay methods used previously it was necessary to establish optimal conditions for DA D1 and D2 receptor binding in rat nucleus accumbens tissue.

6.2 METHODS

6.2.1 Tissue preparation

Male Wistar rats weighing 250-280g were sacrificed by decapitation and the brains rapidly removed, chilled on ice and then sliced coronally with a McIlwain tissue chopper as described in Section 3.2.1. The nucleus accumbens was dissected out of the brain slices which contained this area and stored in liquid nitrogen until assayed. Whole nucleus accumbens tissue was thawed, pooled and homogenized (using a teflon pestle attached to a stirrer, at 900rpm, in a polycarbonate test-tube with a clearance of 0.9mm) in 40 volumes of ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and

1µM pargyline (pH 7 4 at 25° C) according to the method of Billard et al , (1984) The homogenate was centrifuged at 35,000g for 10 minutes and subsequently washed twice with 40 volumes of buffer, then resuspended in sufficient buffer to give 1mg tissue per assay (approximately equivalent to 0 15mg protein/ml) in the final incubation volume except where modifications are stated

6.2.2 DA D1 Receptor assay

Four hundred microlitres of tissue homogenate was pipetted, in duplicate, into polypropylene incubation tubes Twenty microliters of [^{3}H]SCH23390 (diluted in 10% ethanol) was added, resulting in a final concentration of 0 3nM in the total incubation volume of 440µl Total binding was measured in tubes to which a further 20µl of 10% ethanol had been added while nonspecific binding was defined by the inclusion of 20µl of cis-flupenthixol (dissolved in 10% ethanol, 1µ M final concentration) The tubes were incubated at 37° C for 30 minutes The reaction was terminated by the addition of 5ml of ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl 2mM $CaCl_2$, 1mM $MgCl_2$, 0 1% ascorbic acid and 1µ M pargyline (pH 7 4 at 25° C), to each tube followed by rapid vacuum filtration through Schlecher and Schuell No 3362 filters previously soaked in 0 025% Brij Filters were washed twice with 5 ml of ice cold buffer and the radioactivity on the filters was determined by liquid scintillation counting after the addition of 10ml Scintillator 299 (Packard)

6.2.2.1 Incubation time

In order to determine the optimal incubation time, the DA D1 receptor assay was carried out as described in 6 2.2. except that the tubes were incubated at 37° C for 6, 14, 20 or 30 minutes

6.2.2.2 Buffer pH

In order to test the effect of varying buffer pH nucleus accumbens tissue was prepared as described in 6 2 1 After washing the membrane preparation three times in 40 volumes of ice

cold Tris HCl buffer, the preparation was resuspended in ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and 1µM pargyline at a pH of 4.4, 6.8, 7.4, 7.6 or 7.8. Binding conditions for [^{3}H]SCH23390 followed the method described in Section 6.2.2.

6.2.2.3 Displacement experiments

Displacement curves were obtained by substituting 20µl of cis flupenthixol, sulpiride, ketanserin or butaclamol dissolved in 10% ethanol at concentrations ranging from 10pM to 10µM in the final incubation volume for the 1µM cis flupenthixol normally used as displacer in the assay procedure described in Section 6.2.2. A possible additional effect of ketanserin on the specific binding of [^{3}H]SCH23390 was determined by addition of 30nM ketanserin to incubation tubes containing 1µM cis flupenthixol. To determine the proportion of binding which was due to filter binding, 400µl of buffer, instead of tissue homogenate, was added to some of the tubes which did not contain drug. Results were calculated as a percentage of the total binding. IC_{50} values were estimated from plots of percentage binding versus concentration of cis flupenthixol, butaclamol, sulpiride and ketanserin, and were taken as the concentration giving 50% inhibition of specific binding defined by 1µM cis flupenthixol. K_i values were calculated using the equation of Cheng and Prusoff, (1973)

$$K_i = IC_{50}/(1 + [L]/K_D)$$

where [L] was the concentration of labelled ligand, and the K_D was determined experimentally in the saturation experiments described in Section 6.2.2.5.

6.2.2.4 Protein concentration

Nucleus accumbens tissue was prepared as described in Section 6.2.1. After washing the membrane preparation three times in 40 volumes of ice cold 50 mM Tris HCl buffer the tissue was resuspended in sufficient ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM

KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and 1µM pargyline (pH7.4 at 25°C) to give a final concentration of 0.030, 0.075, 0.150, 0.224 or 0.300 mg protein/ml. Binding conditions for [^{3}H]SCH23390 then followed the method described in Section 6.2.2.

6.2.2.5 Saturation experiments

For saturation experiments nucleus accumbens tissue was prepared as described in Section 6.2.1. After washing the membrane preparation three times in 40 volumes of ice cold 50 mM Tris HCl buffer, the tissue was resuspended in sufficient ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1 mM $MgCl_2$, 0.1% ascorbic acid and 1µM pargyline (pH7.4 at 25°C) to give a final concentration of 0.15mg protein/ml. Instead of a single concentration of [^{3}H]SCH23390, 10 concentrations ranging from 0.02-3.0 nM were included in the final incubation volume. To define nonspecific binding, 10µl of cis flupenthixol was added to one set of tubes to give a concentration of 1µM in the final incubation mixture. To prevent [^{3}H]SCH23390 binding to 5-HT_2 binding sites 30nM ketanserin was included in some experiments. The assay was performed as described in Section 6.2.2. Receptor number (B_{max}) and affinity (K_D) values were calculated using the nonlinear least squares curve-fitting program LIGAND described in Section 3.2.6.

6.2.3 DA D2 receptor assay

6.2.3.1 [^{3}H]Spiperone binding

Four hundred microlitres of tissue homogenate was pipetted, in duplicate, into polyproplyene incubation tubes. Twenty microliters of [^{3}H]spiperone (diluted in 10% ethanol) was added, resulting in a final concentration of 0.25nM in the total incubation volume of 440µl. Nonspecific binding was defined by the inclusion of 20µl of sulpiride (dissolved in 10% ethanol, 10µM final concentration) to a second set of tubes. The tubes were incubated at 37° C for 15 minutes. The reaction was terminated by the addition of 5ml of ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and 1µM

pargyline (pH 7 4 at 25° C) to each tube followed by rapid vacuum filtration through Schlecher and Schuell No 3362 filters previously soaked in 0 025% Brij Filters were washed twice with 5 ml of ice cold buffer and the radioactivity on the filters was determined by liquid scintillation counting after the addition of 10ml Scintillator 299 (Packard)

6.2.3.1.1 Incubation time

The assay procedure described in Section 6 2.3 1 was followed except that the tubes were incubated at 37° C for 2, 4, 6, 12 and 20 minutes

6.2.3.1.2. Buffer pH

Nucleus accumbens tissue was prepared as described in Section 6 2 1 After washing the membrane preparation three times in 40 volumes of ice cold 50mM Tris HC buffer, the preparation was resuspended in ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0 1% ascorbic acid and 1µ M pargyline at a pH of 4 4, 6 8, 7 4, 7 6 or 7 8 Binding conditions for [^{3} H]spiperone followed the method described in Section 6.2 3 1

6.2.3.1 3. Displacement experiments

For displacement experiments 20µl of cis flupenthixol, butaclamol, sulpiride or ketanserin dissolved in 10% ethanol at concentrations ranging from 10pM to 10µ M in the final incubation volume was substituted for the 10µ M sulpiride normally used as displacer in the assay procedure described in 6.2.3.1. The additional effect of ketanserin on the specific binding of [^{3} H]spiperone was determined by the inclusion of 3nM ketanserin in incubation tubes containing 10µ M sulpiride To determine the proportion of binding which was due to filter binding, 400µl of buffer, instead of tissue homogenate, was added to tubes which did not contain the displacing drug Results were calculated as a percentage of total binding IC_{50} and K_i values were obtained as described for [^{3} H]SCH23390 displacement experiments in Section

6.2.2 3

6.2.3.1.4. Protein concentration

Nucleus accumbens tissue was prepared as described in Section 6.2 1 After washing the membrane preparation three times in 40 volumes of ice cold 50 mM Tris HCl buffer, the tissue was resuspended in sufficient ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1 mM $MgCl_2$, 0 1% ascorbic acid and 1µ M pargyline (pH 7 4 at 25° C) to give a final concentration of 0 030, 0 075, 0 150, 0 224 and 0 300 mg protein/ml Binding conditions for [^{3}H]spiperone then followed the method described in Section 6 2 3 1

6.2.3.1 5 Saturation experiments

Nucleus accumbens tissue was prepared as described in Section 6 2 1 After washing the membrane preparation three times in 40 volumes of ice cold 50 mM Tris HCl buffer, the tissue was resuspended in sufficient ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0 1% ascorbic acid and 1µ M pargyline (pH7 4 at 25° C) to give a final concentration of 0 05mg protein/ml. For saturation experiments, 10 concentrations of [^{3}H]spiperone ranging from 0 04-2 0nM were included in the final incubation volume To define nonspecific binding, 10µl of sulpiride was added to give a concentration of 10µ M in the final incubation mixture. To prevent [^{3}H]spiperone binding to 5-HT_3 receptor binding sites 3nM ketanserin was included in some experiments The assay was performed as described in Section 6 2.3 1 Receptor number (Bmax) and affinity (K_D) values were calculated using the nonlinear least squares curve-fitting program LIGAND as described in Section 3 2 6.

6.2.3.2 [^{125}I]Iodospiperone binding

6.2.3.2.1 Displacement experiments

Four hundred microlitres of tissue homogenate was pipetted, in duplicate, into incubation tubes and 20µl of [^{125}I]iodospiperone (diluted in 10% ethanol) was added to produce a

concentration of 0 2nM in the final assay volume of 440µl 20µl of sulpiride at a concentration range of 0 1nM to 0.1mM, or 20µl of butaclamol at a concentration r-- of 10pM to 10µ M were added as displacers for the radioligand Total binding was measured, in triplicate for each drug, in tubes containing 20µl of 10% ethanol instead of the displacing drug The samples were incubated at 37° C for 15 minutes. To determine the proportion of binding which was due to filter binding, 400µl of buffer, instead of tissue homogenate, was added to tubes which did not contain the displacing drug The reaction was terminated by the addition of 5ml ice cold 50mM Tris HCl buffer followed by rapid vacuum filtration through Schlecher and Schuell No 3362 filters previously soaked in 0 025% Brij, 10µ M spiperone, or a combination of the two Filters were washed two or three times with 5ml of ice cold 50mM Tris HCl buffer, placed in polycarbonate test tubes and the radioactivity remaining on the filters was measured in a LKB Wallace 1260 Multigamma gamma counter Results were calculated as a percentage of total binding and plotted versus concentration of sulpiride or butaclamol

6.3 RESULTS

6.3.1 Effect of incubation time on [^{3}H]SCH23390 binding

The specific binding of [^{3}H]SCH23390 (0 3nM) to nucleus accumbens homogenate increased time dependently and appeared to reach equilibrium after 25 minutes of incubation at 37° C (Fig 6 1).

6.3.2. Effect of buffer pH on [^{3}H]SCH23390 binding

The specific binding of [^{3}H]SCH23390 was relatively independent of increases in pH up to 7 4, but at higher pH levels binding was observed to decrease markedly (Fig 6 2)

6.3.3. Displacement of [^{3}H]SCH23390 binding

Displacement curves were generated for cis flupenthixol, butaclamol, sulpiride and ketanserin using [^{3}H]SCH23390 as radioligand (Fig 6 3) The total binding of 0 3 nM [^{3}H]SCH23390

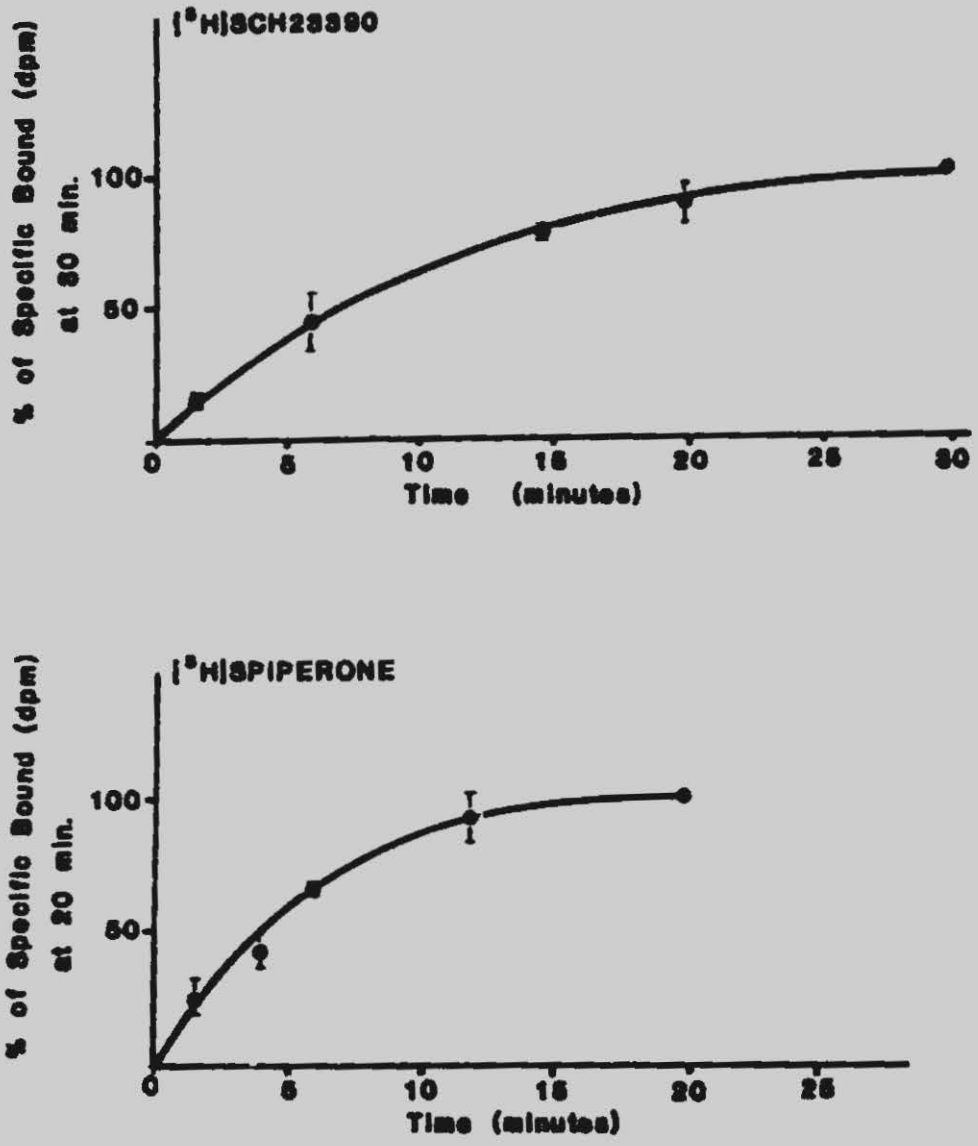

Fig. 6.1 Effect of incubation time on specific binding of 0.3nM [³H]SCH23390 and 0.25nM [³H]spiperone in the nucleus accumbens of the rat. Results were calculated as a percentage of the mean specific binding at 30minutes and 20 minutes for [³H]SCH23390 and [³H]spiperone respectively. Means ± SEM of 3 experiments in duplicate are shown. At equilibrium specific binding was 4097dpm and 1483 dpm for [³H]SCH23390 and [³H]spiperone respectively.

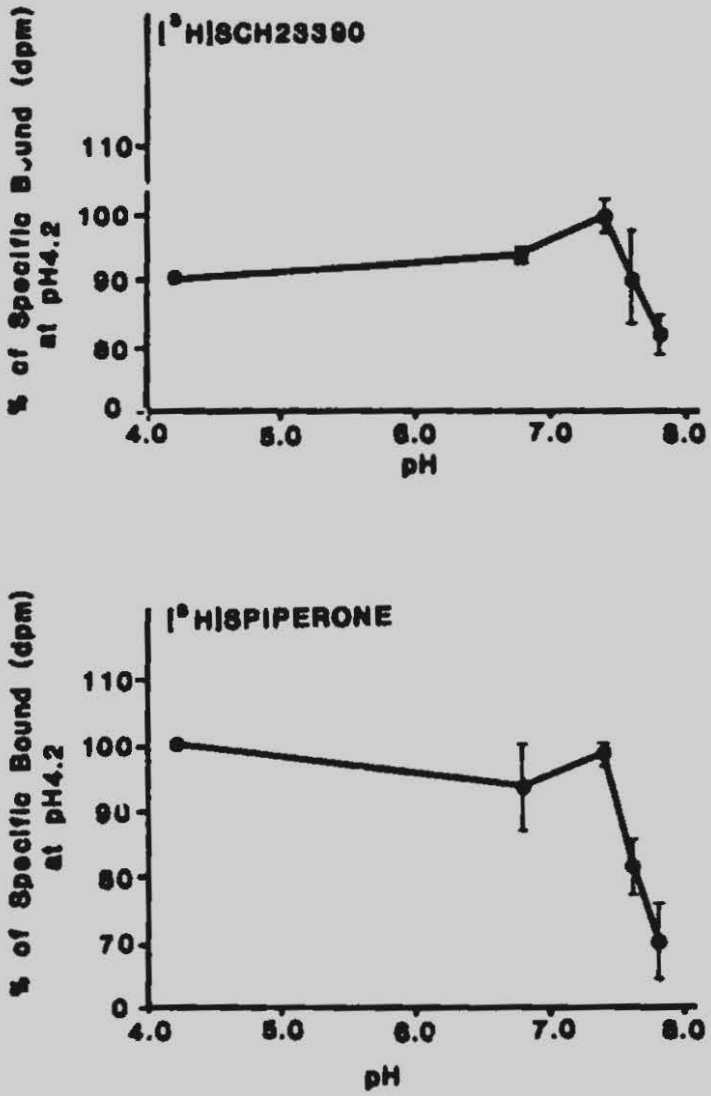

Fig. 8.2 Effect of buffer pH on specific binding of 0.3nM [³H]SCH23390 and 0.25nM [³H]spiperone in the nucleus accumbens of the rat. Results were calculated as a percentage of the mean specific binding observed at pH 4.2. Means ± SEM of 3 experiments in cuplicate are shown.

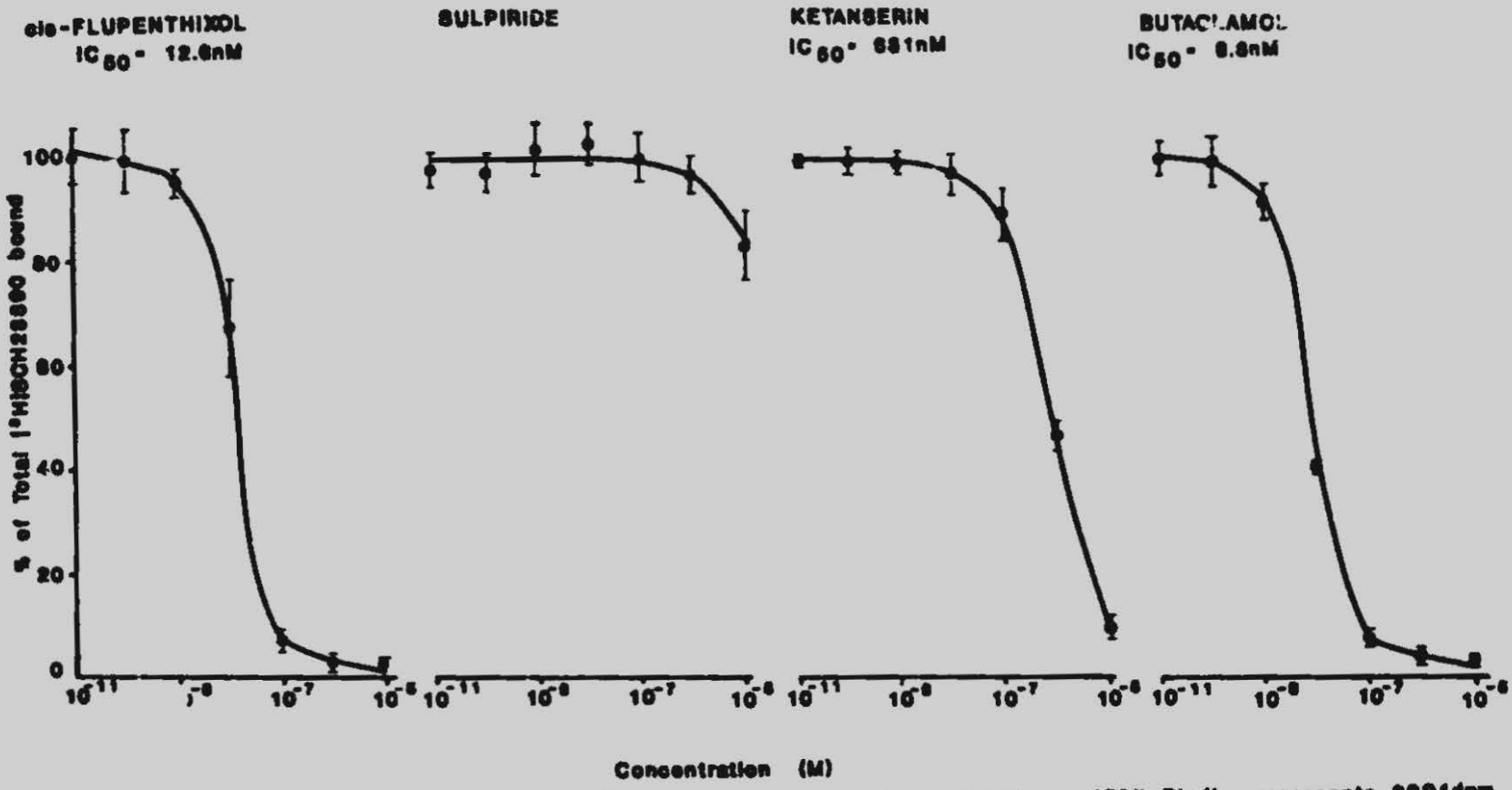

Fig. 6.8 Drug displacement curves of 0.9nM [^{3}H]SCH23390 in rat nucleus accumbens. 100% Binding represents 6991dpm. Mean ± SEM of 3 experiments in duplicate are shown.

was displaced 98% by 1µ M cis-flupenthixol, 97% by 1µ M butaclamol and 90% by 10µ M ketanserin No displacement was observed with sulpiride up to concentrations of 10µ M. When the 5-HT_2 antagonist ketanserin was included in the incubation tubes where maximal displacement of [^{3}H]SCH23390 by 1µ M cis-flupenthixol had taken place, no further displacement was observed. Approximations of IC_{50} and K_i values for cis-flupenthixol, butaclamol, and ketanserin are shown in Table 6 1 Nonspecific binding of [^{3}H]SCH23390 (defined by the presence of 1µ M cis-flupenthixol) was found to be 2-6% of the total binding and filter binding was 2-3% of total binding

6.3.4. Effect of protein concentration on [^{3}H]SCH23390 binding

Specific binding of [^{3}H]SCH23390 was found to be a linear function of protein concentration over the entire range examined (up to 0.3mg protein/ml), Fig 6 4

6.3.5 Saturation curves for [^{3}H]SCH23390 and the effect of ketanserin.

The specific binding of [^{3}H]SCH23390 (0.02-3 0nM) was saturable (Fig. 6 5) whereas nonspecific binding increased linearly over the concentration range examined. Receptor number (Bmax) and affinity (K_D) for [^{3}H]SCH23390 binding in the nucleus accumbens, measured with and without the addition of 30nM ketanserin to mask 5-HT_2 binding sites, are shown in Table 6 2. For each saturation experiment the best fit by LIGAND was by a one site model. No differences were observed in values of Bmax or K_D measured in the presence or absence of 30nM ketanserin.

3.3.6 Effect of incubation time on [^{3}H]spiperone binding

The specific binding of [^{3}H]spiperone (0.25 nM, at 37° C in nucleus accumbens tissue homogenate was rapid and reached a maximum after 15 minutes (Fig 6 1). The specific binding of [^{3}H]spiperone at equilibrium amounted to 1433 dpm, 2.8 times lower than the specific binding of [^{3}H]SCH23390 which was 4097 dpm at equilibrium.

TABLE 6.1

Drug inhibition of [^{3}H]SCH23390 and [^{3}H]SPIPERONE binding in rat nucleus accumbens.

	RADIOLIGAND			
	[^{3}H]SCH23390		[^{3}H]SPIPERONE	
Displacing drug	IC_{50} (nM)	K_i (nM)	IC_{50} (nM)	K_i (nM)
cis-Flupenthixol	12 6	6.9	19.9	3.3
Sulpiride	>10000	5555	199.5	33 2
Ketanserin	631	344 2	600	100
Butaclamol	6.3	3 4	2 8	0 5

IC_{50} and K_i values are expressed in nM. K_i values were obtained using the Cheng and Prusoff, (1973) equation as described in Section 6.2 2 3. Estimations of IC_{50} values were made from Figs. 6.3 and 6.6.

6.3.7 Effect of buffer pH on [^{3}H]spiperone binding

The specific binding of [^{3}H]spiperone to nucleus accumbens membranes was found to be optimum at pH 7.4 (Fig 6 2), and as was found with [^{3}H]SCH23390, decreased rapidly in buffers with pH higher than 7 4

6.3.3 Displacement of [^{3}H]spiperone by antagonists

The total binding of 0.25nM [^{3}H]spiperone was displaced 86% by 1 M cis-flupenthixol; 90% by 1µ M butaclamol and 74% by 10µ M sulpiride (Fig.6.6). Displacement of [^{3}H]spiperone by ketanserin appeared to be biphasic, with a possible initial displacement between 1 and 10CnM ketanserin and a subsequent displacement of 85% of the total [^{3}H]spiperone binding by 10µ M ketanserin. Displacement of [^{3}H]spiperone by 10µ M sulpiride was slightly increased 1.7-3 8% by the addition of 3nM ketanserin The nonspecific binding of [^{3}H]spiperone, measured in the presence of 10µ M sulpiride, was found to be 16 24% of total binding, filter

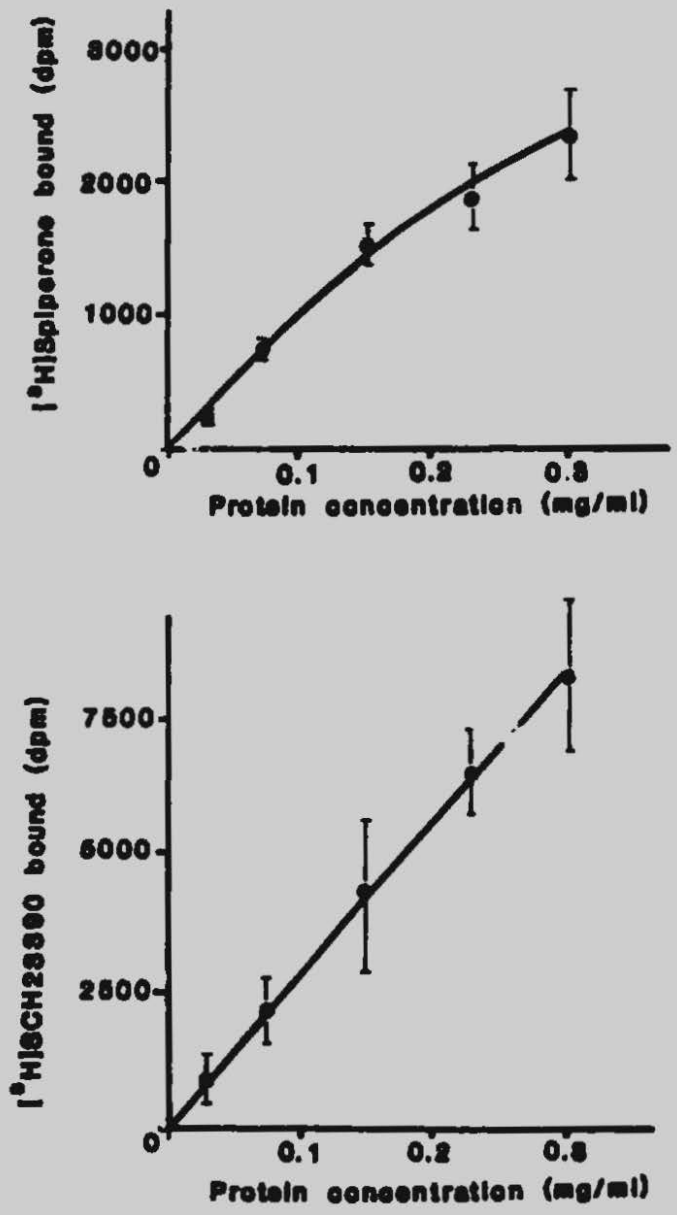

Fig. 6.4 Effect of protein concentration on the specific binding of 0.3nM [^{3}H]SCH23390 and 0.25nM [^{3}H]spiperone in the nucleus accumbens of the rat. Means ± SEM of 8 experiments in duplicate are shown.

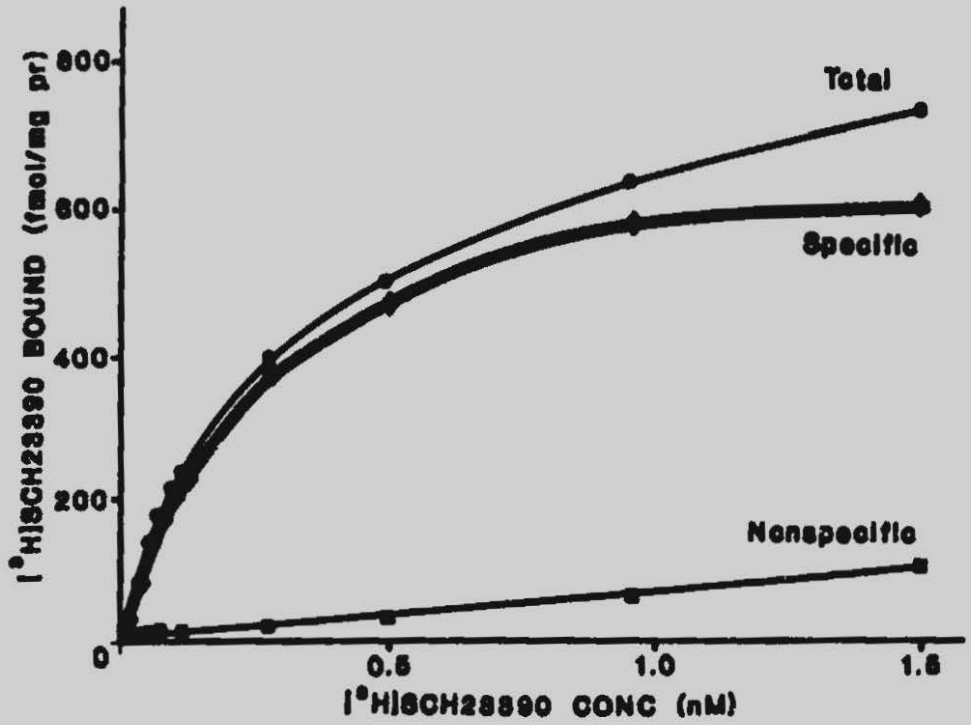

Fig. 6.5 Saturation of specific [^{3}H]SCH23390 binding in rat nucleus accumbens. Specific binding was defined as the difference between total binding and nonspecific binding in the presence of 1μM cis-flupenthixol.

TABLE 6.2

Effect of ketanserin on the receptor number (Bmax) and affinity (K_D) of DA D1 and D2 receptors

	[^{3}H]SCH23390 binding		[^{3}H]SPIPERONE binding	
	Bmax (fmol/mg protein)	K_D (nM)	Bmax (fmol/mg protein)	K_D (nM)
without ketanserin	560.0 644.7	0.360 0.391	127 7 124.0	0.048 0.046
with ketanserin	580.8 660.7	0.440 0.439	129 3 95.6	0.035 0.026

Results shown are from individual experiments 30nM Ketanserin was included in the [^{3}H]SCH23390 binding assay and 3nM ketanserin in the [^{3}H]SPIPERONE binding assay.

binding was 9-10% of total binding. IC_{50} and K_i values for cis-flupenthixol, butaclamol, and sulpiride were obtained by measurement fro n Fig. 6.6 and are shown in Table 6.1.

6.3.9 Effect of protein concentration on [^{3}H]spiperone binding

Specific binding of [^{3}H]spiperone was linear up to a protein concentration of 0.2mg/ml (Fig 6.4). At higher protein concentrations specific binding of [^{3}H]spiperone was observed to decrease

6.3.10 Saturation curves for [^{3}H]spiperone and the effect of ketanserin

Equilibrium saturation binding showed that the specific binding of [^{3}H]spiperone was saturated over the concentration range used (0.4-2nM) while nonspecific binding increased linearly with increasing concentrations of [^{3}H]spiperone (Fig. 6.7). The best fit by LIGAND was obtained using a one site model and the receptor number (Bmax) and affinity K_D thus estimated are shown in Table 6.1. The estimations of Bmax measured in the presence of 3nM ketanserin (124 and 95 fmol/mg protein) were not apparently different from those measured without the addition of ketanserin (127 and 129 fmol/mg protein).

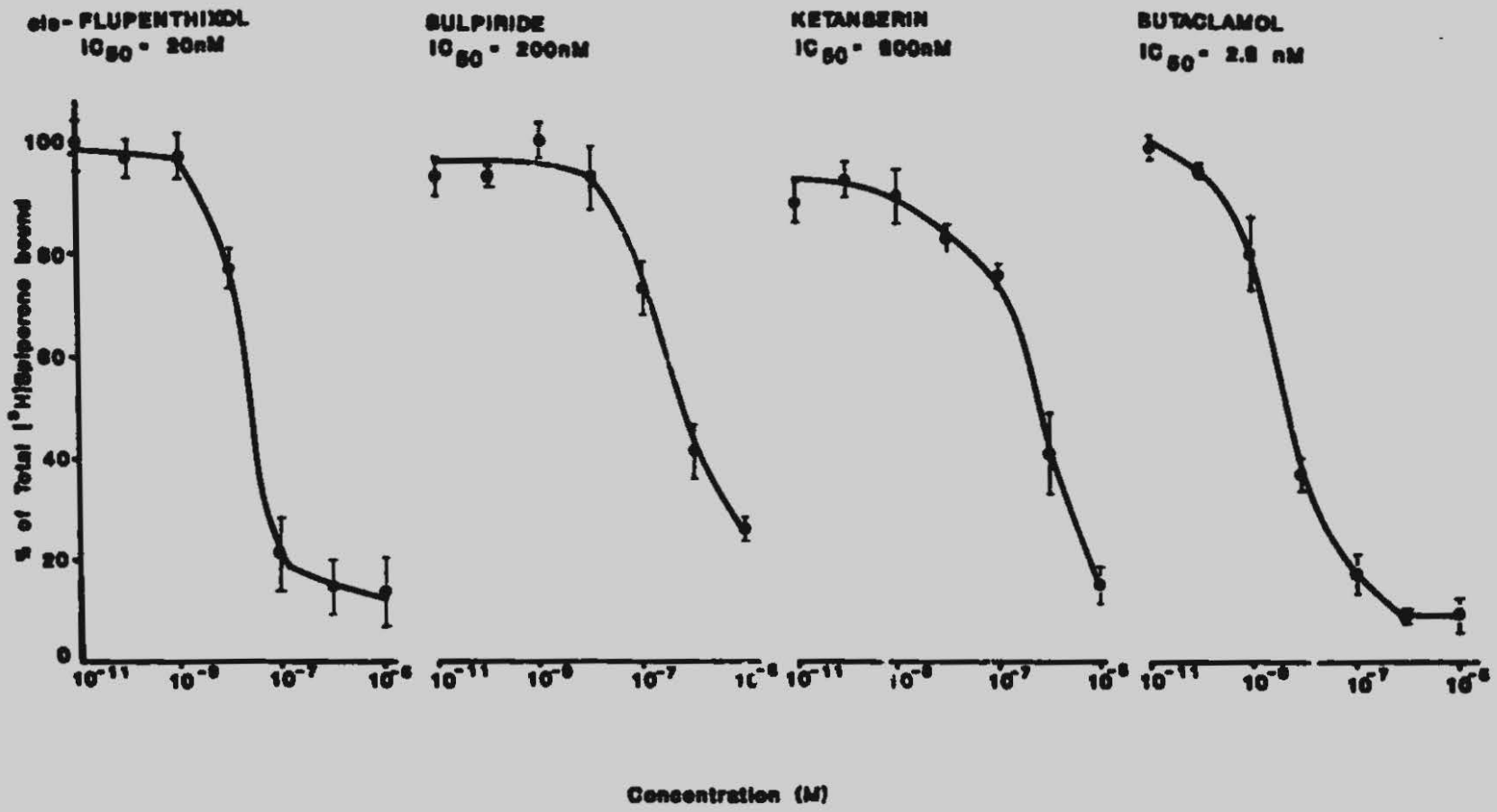

Fig. 6.8 Drug displacement curves of 0.25nM [^{3}H]spiperone in rat nucleus accumbens. 100% Binding represents 1246dpm. Mean ± SEM of 8 experiments in duplicate are shown.

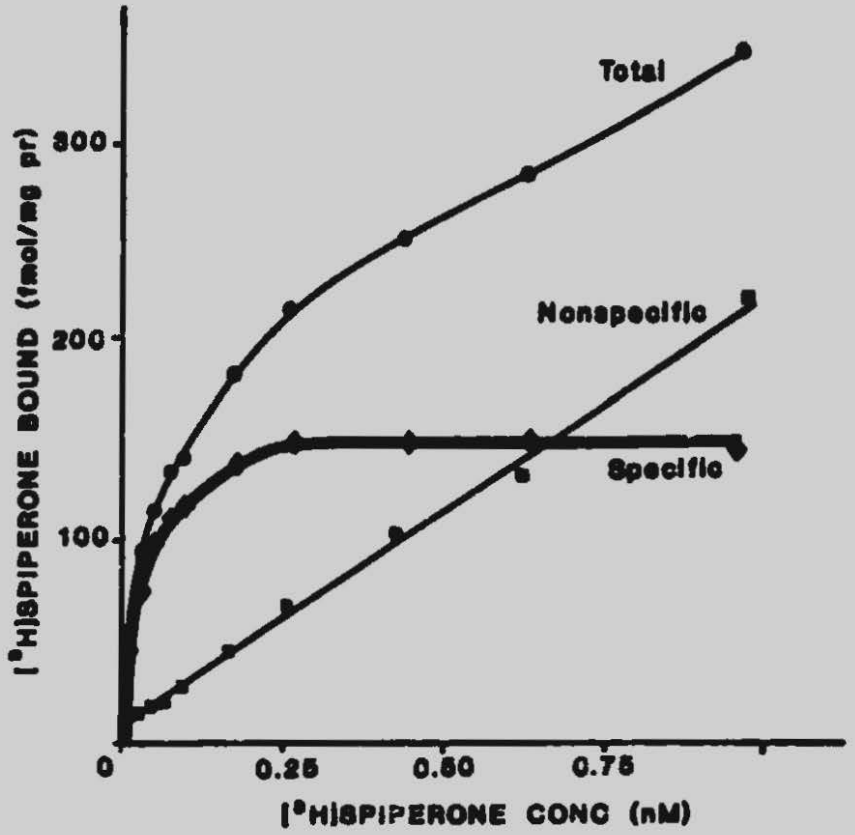

Fig. 6.7 Saturation of specific [^{3}H]spiperone binding in rat nucleus accumbens. Specific binding was defined as the difference between total binding and nonspecific binding in the presence of 10µM sulpiride.

6.3.11 [^{125}I]Iodospiperone binding

Rather shallow displacement curves for sulpiride and butaclamol were obtained with [^{125}I]iodospiperone binding (Fig 6.8). [^{125}I]Iodospiperone binding was displaced 28% by 10μM sulpiride and 47% by 10μM butaclamol. Nonspecific binding (measured in the presence of 10μM sulpiride) was found to be extremely high, 65-75% of total binding and filter binding was 10-30% of total binding. Attempts to reduce filter binding by soaking filters in solutions of 0.025% Brij, 10μM spiperone, or a combination of the two, or by increasing filter washing were without success.

6.4 DISCUSSION

DA D1 receptor assays using [^{3}H]SCH23390 as radioligand have been carried out by several workers (Kilpatrick et al., 1986; Hyttel and Arnt, 1987; Schulz et al., 1985a; Billard et al., 1984), in which the stereoselectivity of [^{3}H]SCH23390 has been clearly established (Kilpatrick et al., 1986; Schulz et al., 1985, Billard et al., 1984). Billard et al., (1984) reported the R-enantiomer, SCH23390, to be 640 times more potent than the S-enantiomer, SCH23388. Therefore in this study it has been accepted that the binding of [^{3}H]SCH23390 is stereoselective. Similarly, the physiological activity has been established by displacement with thioxanthenes such as cis-flupenthixol and pifluthixol, which have been reported to have a high affinity for both DA D1 and D2 receptors (Hyttel and Arnt, 1987; Hyttel and Arnt, 1986) and which have been used as DA D1 receptor ligands, in the presence of DA D2 selective antagonists such as sulpiride, and by its antagonist effect on DA stimulated adenylate cyclase (Hyttel, 1983; Itoh et al., 1984; Andersen et al., 1985). The chemical purity of [^{3}H]SCH23390 was established by thin layer chromatography (Section 3.2.4), and it was shown to be 98.6% pure. Various buffer compositions have been used in DA D1 assays and the method of Billard et al., (1984) was adopted for the present study, using 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and 1μM pargyline (pH7.4 at

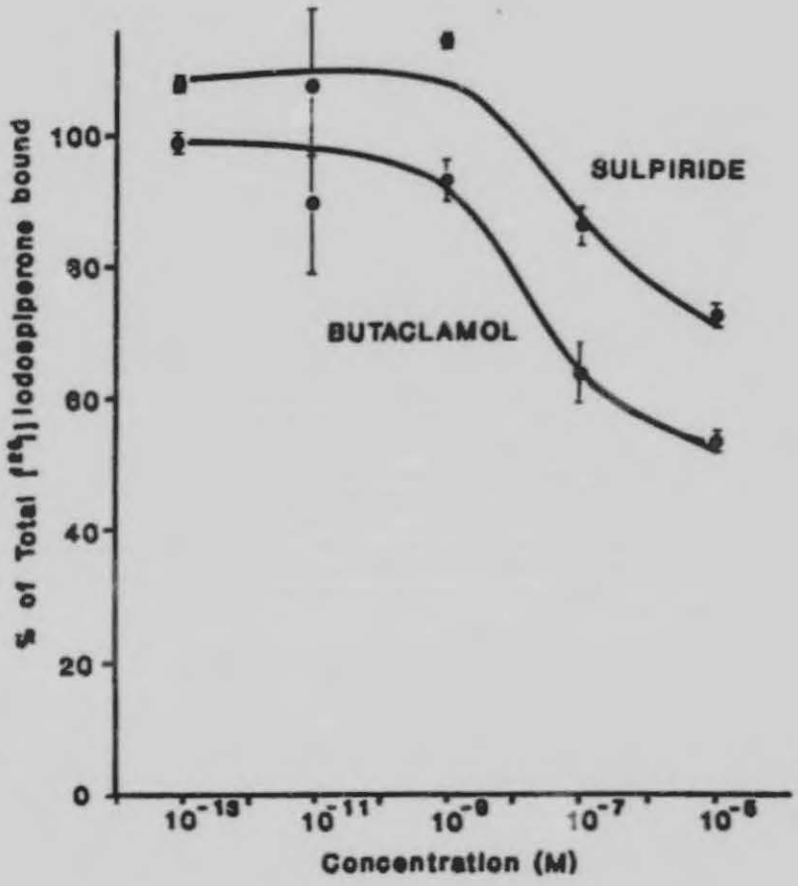

Fig. 6.8 Drug displacement curves of 0.2nM [^{125}I] iodospiperone in rat nucleus accumbens. 100% Binding represents 35497cpm. Means ± SEM of 3 observations are shown.

25° C). In previous studies it has been shown that the specific binding of antagonists is enhanced by the inclusion of the above mentioned cations as these appear to delay the dissociation of the radioligand from the receptor (Hyttel and Arnt, 1987). Ascorbic acid (0.1%) and pargyline (1μ M) were included to retard nonspecific and enzyme catalysed oxidation of sensitive compounds.

The conditions for equilibrium binding in the nucleus accumbens were determined so that saturation experiments could be carried out at steady state. Under the present conditions the specific binding of [^{3}H]SCH23390 appeared to have reached equilibrium relatively rapidly, after 20-30 minutes at 37° C (Fig 6.1). This finding is in agreement with other authors. Kilpatrick et al., (1986) and Schulz et al., (1985a) reported equilibrium of [^{3}H]SCH23390 binding in the striatum to be reached after 15 minutes at 37° C. Therefore in subsequent experiments an incubation time of 30 minutes at 37° C was used for [^{3}H]SCH23390 binding. Many workers have conducted [^{3}H]SCH23390 binding assays in the physiological buffer pH of 7.4 (Billard et al., 1984; Kilpatrick et al., 1986; Klimek and Nielsen, 1987). The present results provide evidence that specific binding of [^{3}H]SCH23390 was optimal at this pH and are therefore in agreement with these authors.

However one study has shown contradictory evidence, reporting the optimum buffer pH for [^{3}H]SCH23390 binding in striatum to be 7.8-8.0 (Schulz et al., 1985a) and Hyttel and Arnt, (1987) have reported [^{3}H]SCH23390 binding to be independent of pH in the range 6.8-7.8. It was noted in the present study that binding decreased sharply at pH levels greater than 7.4 and care was taken to ensure that the pH did not exceed this value.

Increases in protein concentration have been reported to decrease the affinity of radioligands for receptor sites (Leysen, 1980), therefore the effect of protein concentration on [^{3}H]SCH23390 binding was studied. Specific binding of [^{3}H]SCH23390 at a concentration of 0.3nM was shown to be linear over a protein concentration range of 0.03-0.30mg protein/ml. For saturation analyses in this study a protein concentration as close to 0.05mg/ml in the final

incubation volume was used so that changes in binding parameters could not be attributed to the influence of protein concentration.

The specific binding of [^{3}H]SCH23390 was displaced with high affinity by cis-flupenthixol and butaclamol. Because IC_{50} values depend on the concentration of ligand, and amount of tissue used in the assay it is not possible to compare values obtained in different laboratories where different assay conditions are used. K_i values were therefore calculated as previously described (Cheng and Prusoff, 1973). K_i values in the nanomolar range, 6.9 and 3.4 nM, were obtained for cis-flupenthixol and butaclamol respectively. This is in agreement with other reports. Hyttel and Arnt, (1987) and Billard et al., (1984) reported K_i values of 1.0 and 4.3 nM respectively for cis-flupenthixol in rat striatum while O'Boyle and Waddington, (1987) obtained a K_i of 15.5nM for cis-flupenthixol in human caudate nucleus. K_i values of 2.1 and 14.6 nM for butaclamol were reported by Hyttel and Arnt, (1987) and Billard et al., (1984) respectively and 3.4 nM for butaclamol was reported by O'Boyle and Waddington, (1987). The selective DA D2 antagonist sulpiride did not displace [^{3}H]SCH23390 up to a concentration of 10μ M. A similar lack of activity has been widely reported for the displacement of [^{3}H]SCH23390 by sulpiride (O'Boyle and Waddington, 1987; Hyttel and Arnt, 1987; Billard et al., 1984; Andersen and Nielsen, 1986). These results confirm the selectivity of [^{3}H]SCH23390 for DA D1 receptor sites. In agreement with other authors (Kilpatrick et al. 1986; Hyttel and Arnt, 1987; Billard et al., 1984) the nonspecific binding was found to be very low; less than 10% of the total binding, and these properties indicate that [^{3}H]SCH23390 is an excellent ligand for the study of DA D1 receptors.

In the present study ketanserin displaced [^{3}H]SCH23390 in nucleus accumbens tissue with relatively low affinity (Fig. 6.3, Table 6.1). The K_i was 344nM, which compares favourably with the value (205 nM) reported by Andersen and Nielsen (1986) and the value (160 nM) obtained by Hyttel and Arnt (1987), both measured in striatal tissue. SCH23390 has been reported to interact with 5-HT_1 (Skarsfeldt and Larsen, 1988) and 5-HT_2 receptor sites

(Bischoff et al., 1986, Bischoff et al., 1988, Closse et al., 1984) Bischoff et al., (1986) have reported that SCH23390 competes with [^{3}H]ketanserin with an IC_{50} value of 30nM in frontal cortex and striatal tissue (Bischoff et al., 1988) and with the 5-HT_1 site (using [^{3}H]5-HT as radioligand) with micromolar affinity These findings indicate that there may be 5-HT_1 and 5-HT_2 components in the binding of [^{3}H]SCH23390 In saturation experiments the highest concentration of he [^{3}H]SCH23390 used is 3.0 nM and it is therefore unlikely that there will be interference by 5-HT_1 receptor binding at this concentration. In DA D1 receptor assays using [^{3}H]SCH23390 as radioligand Klimek and Nielsen, (1987) have shown that in striatal tissue the presence of 40nM ketanserin significantly decreased the specific binding of [^{3}H]SCH23390 and 30-40nM ketanserin has been included routinely in assays to mask 5 HT_2 binding (Bischoff et al., 1986; Porceddu et al., 1987) Cis flupenthixol has been reported to have some affinity for 5-HT_2 receptors, demonstrated by displacement from [^{3}H]spiperone in the rat frontal cortex but appears to be relatively inactive at 5-HT_1 sites (Closse et al., 1984) The displacement of [^{3}H]SCH23390 by cis-flupenthixol in this study may therefore be due to both DA D1 and 5 HT_2 receptor sites Results of the present study show that [^{3}H]SCH23390 binding, after maximal displacement by 1 µ M flupenthixol, was not further decreased by the addition of 30nM ketanserin In addition, the inclusion of 30nM ketanserin did no appear to alter the maximal number of binding sites (Bmax) or affinity of receptors measured in the saturation experiments (Table 6 5) and [^{3}H]SCH23390 was found to bind to a single site Nevertheless, as the presence of both 5-HT_1 and 5-HT_2 receptors has been shown in the nucleus accumbens by quantitative autoradiographic studies (Pazos and Palacios, 1985, Pazos et al., 1985) any possible interference by 5-HT_2 receptors in the [^{3}H]SCH23390 binding assay was excluded by the inclusion of 30nM ketanserin throughout

Under the present conditions [^{3}H]SCH23390 binds with high affinity to a single population of receptor sites in the nucleus accumbens The receptor density (560-660 fmol/mg protein) is similar to that previously reported for nucleus accumbens membrane preparations by Hyttel and Arnt (1987) who measured a Bmax of 810 fmol/mg protein Dubois et al., (1986) measured

the DA D1 receptor density in the nucleus accumbens using the DA D1 selective agonist, [^{3}H]SKF 38393, in an autoradiographic study and obtained 727 fmol/mg protein. Autoradiographic studies using other DA D1 receptor ligands have resulted in many differing values. Savasta et al., (1987a) reported a value of 2458 fmol/mg protein using [^{3}H]SCH23390, Barone et al., (1987) obtained a value of 58 fmol/mg protein and Aiso et al., (1987) obtained a value of 175 fmol/mg protein. K_D values of [^{3}H]SCH23390 have been shown to be similar in various brain areas The K_D obtained in the present study (0.36-0.44nM) agrees with those previously reported for striatum and nucleus accumbens tissue Kilpatrick et al., (1986) and Billard et al., (1984) reported values of 0 36 and 0.53 nM respectively for striatal tissue, while Klimek and Nielsen, (1987) reported a value of 0 32nM for nucleus accumbens tissue.

The present results provide evidence that [^{3}H]SCH23390 is a selective ligand for DA D1 receptors with high affinity and low nonspecific binding and therefore appears to be a suitable ligand for studying these receptors in the nucleus accumbens of the rat

The methods of Leysen and Gommeren, (1981) and Creese et al., (1977) were adopted as the starting point for characterizing DA D2 sites using the ligand [^{3}H]spiperone The stereoselectivity of spiperone has been well established (Leysen and Gommeren, 1981, Creese et al., 1977, Closse et al., 1984, Hyttel and Arnt, 1986), (+)butaclamol being about 1000 times more potent than its stereoisomer (-) butaclamol (Fields et al., 1977). The physiological activity of neuroleptics has also been studied extensively (Howlett and Nahorski, 1980; Urwyler and Coward, 1987; Closse et al., 1984; Leysen et al., 1978) and spiperone has been reported to be "a ligand of choice for neuroleptic receptors" (Laduron et al., 1978) As with [^{3}H]SCH23390 the chemical purity of [^{3}H]spiperone was established by thin layer chromatography (Section 3.2 4) and it was found to be 95-98% pure. Variations in assay conditions such as buffer pH and composition have been shown to alter specific binding of [^{3}H]spiperone (Leysen and Gommeren, 1981, List and Seeman, 1981; Leysen, 1980) and, as was reported for [^{3}H]SCH23390 binding, monovalent and divalent cations increase the specific antagonist

binding of [^{3}H]spiperone by retarding its dissociation from membrane binding sites (Creese et al., 1978; Usdin et al., 1980). Therefore 120mM NaCl, 5mM KCl, 2mM $CaCl_2$ and 1mM $MgCl_2$ were included in the Tris HCl buffer as well as 0.1% ascorbic acid and 1μM pargyline. Leysen and Gommeren, (1981) have previously shown that [^{3}H]spiperone binding is optimal in this Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and 1μM pargyline.

[^{3}H]Spiperone binding in 50mM Tris HCl buffer (pH7.4 at 25° C) containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and 1μM pargyline reached equilibrium after 15 minutes incubation at 37° C (Fig 6.1). This is in agreement with many authors (Leysen and Gommeren, 1981; Klimek and Nielsen, 1987; Usdin et al., 1980) who use these incubation conditions for [^{3}H]spiperone binding. The optimal buffer pH was found to be 7.4, as was the case for [^{3}H]SCH23390 binding. The specific binding of [^{3}H]spiperone also decreased rapidly at pH values greater than 7.4 and care was therefore taken that the pH of the buffer did not exceed 7.4 for either ligand. This is in agreement with some authors (List and Seeman, 1981; Andersen et al., 1985; Urwyler and Coward, 1987) but in other studies buffers of higher pH have been used. Leysen, (1980) has reported pH 7.5 to be optimal, and pH 7.7 was used by Usdin et al., (1980) and Hamblin et al., (1984) for [^{3}H]spiperone binding assays. These differences could be due to variation in conditions between laboratories. Unlike [^{3}H]SCH23390 binding, [^{3}H]spiperone binding was only found to be linear with protein concentrations up to 0.19mg protein/ml, above which value the specific binding of [^{3}H]spiperone was inhibited. A limited linear dependence on protein concentration for [^{3}H]spiperone binding has also been reported by Leonard et al., (1987) who reported [^{3}H]spiperone binding to increase linearly up to a protein concentration of 0.5mg/ml. K_D values are dependent on tissue concentration (Cheng and Prusoff, 1973), therefore binding was carried out within the linear range. It was necessary to use as much tissue as possible so that low concentrations of receptors in some areas of the nucleus accumbens could be measured. Protein concentrations as close to 0.15mg protein/ml as possible were used throughout.

The total binding of [^{3}H]spiperone was displaced with high affinity by cis-flupenthixol and butaclamol, drugs which are active at both DA D1 and D2 sites (Hyttel and Arnt, 1986, Billard et al., 1984). A K_i value of 2.6nM was calculated for cis-flupenthixol and this is within the range (0.74-3.2 nM) of values previously reported (Billard et al., 1984, Hyttel, 1983, Hyttel and Arnt, 1986). The K_i obtained for butaclamol was 0.83nM which is also similar to those previously reported. Billard et al., (1984) obtained a value of 1.8nM and Urwyler and Coward (1987) reported a value of 4.1nM. [^{3}H]Spiperone binding was displaced with somewhat lower affinity by sulpiride. The K_i was 33.2nM. This is in agreement with other reports of K_i values between 6.8 and 150nM (Urwyler and Coward, 1987, Billard et al., 1984; List and Seeman, 1981). Sulpiride has been described as a selective DA D2 antagonist (O'Connor and Brown, 1982; Kebabian and Calne, 1979; List and Seeman, 1981; Hamblin et al., 1984) and in the present study has been shown to displace 74% of the total [^{3}H]spiperone binding at a concentration of 10μM. It does not displace [^{3}H]SCH23390 binding at this concentration and therefore appears to be selective for DA D2 receptor sites. Nonspecific binding was found to be 16-24% of the total binding which is sufficiently low to allow accurate measurement of receptor parameters.

Ketanserin displaced [^{3}H]spiperone (0.25nM) in a biphasic manner (Fig 6.8). Accurate measurement of the IC_{50} was not possible from the graph obtained. A similar displacement curve was obtained by Hamblin et al., (1984) who reported K_i values of 0.33nM and 740nM for the competitive inhibition of 0.1nM [^{3}H]spiperone by ketanserin. Billard et al., (1984) reported a value of 397nM which seems to correspond to the low affinity value observed in this study. The high affinity site has been suggested to represent displacement from 5-HT_2 sites by ketanserin while the low affinity site represents ketanserin displacement of [^{3}H]spiperone from DA D2 sites (Hamblin et al., 1984). After maximal displacement of binding to DA D2 receptors by 10μM sulpiride, [^{3}H]spiperone binding was further decreased by 1.7-3.8% of the total binding after the addition of 3nM ketanserin. This also suggests that a proportion of the total

[^{3}H]spiperone binding measured is due to 5-HT_2 receptors. There has been evidence that the inclusion of 5-HT_2 antagonists such as ketanserin, cinanserin and R43448 in the incubation medium successfully masks binding to 5-HT_2 receptors (Leonard et al., 1987, List and Seeman, 1981; Hamblin et al., 1986) therefore 3nM ketanserin was included for this purpose in all subsequent [^{3}H]spiperone binding experiments.

The specific binding of [^{3}H]spiperone was shown to be saturable and to occupy a single receptor site in the nucleus accumbens, with or without the inclusion of ketanserin as demonstrated by the best fit by LIGAND. The Bmax values, 127.7 and 129.3 fmol/mg protein, measured without the addition of ketanserin appeared to be slightly higher than the values obtained when ketanserin was added (124 0 and 95 6 fmol/mg protein) This decrease could be due to the elimination of 5-HT_2 binding by the addition of ketanserin. However, more definite evidence for the involvement of 5-HT_2 receptors in the binding of [^{3}H]spiperone was shown in the displacement experiments and 3nM ketanserin was added routinely throughout. The receptor density, approximately 125 fmol/mg protein is similar to that found by Richfield et al., (1986) who found DA D2 receptor density to be 112 fmol/mg protein using [^{3}H]spiperone in the presence of mianserin Dubois et al., (1986) used [^{3}H]NPA in an autoradiographic study to measure the density of DA D2 receptors in the nucleus accumbens and obtained a value of 255 fmol/mg protein. In another study the DA D2 agonist [^{3}H]CV 205-502 was used and a value of 224 fmol/mg protein was reported (van der Weide et al., 1987b).

[^{125}I]Iodospiperone showed unacceptably high nonspecific binding levels under the conditions used, and was therefore rejected as a suitable ligand for DA D2 receptors. These results indicate that under the present conditions the binding of [^{3}H]SCH23390 to DA D1 receptors and [^{3}H]spiperone to DA D2 receptors is saturable and of high affinity and selectivity when 1µ M cis-flupenthixol is used as nonspecific displacer for [^{3}H]SCH23390 and when 10µ M sulpiride in the presence of 3nM ketanserin is used for [^{3}H]spiperone binding Binding equilibrium was reached rapidly for both ligands and nonspecific binding was sufficiently low

to allow accurate measurement of specific binding [^{3}H]SCH23390 was observed to bind to 4 times as many receptors in the nucleus accumbens as [^{3}H]spiperone This is similar to the findings of Hyttel and Arnt, (1986) who have reported 2-3 times as many DA D1 as DA D2 receptors in the striatum and an increasing D1/D2 ratio from the striatum through the nucleus accumbens to the olfactory tubercle. Therefore under the present conditions [^{3}H]SCH23390 and [^{3}H]spiperone have been accepted as suitable ligands for DA D1 and D2 receptors respectively.

6.5 SUMMARY

Optimal binding conditions for DA D1 receptors using [^{3}H]SCH23390 and DA D2 receptors using [^{3}H]spiperone were determined for rat nucleus accumbens The specific binding of [^{3}H]SCH23390 and [^{3}H]spiperone was dependant on incubation time, buffer pH and protein concentration The specific binding of [^{3}H]SCH23390 and [^{3}H]spiperone was optimal in 50mM Tris HCl buffer at pH 7.4 (at 25° C) containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and 1μ M pargyline. Equilibrium was reached after 30 minutes for [^{3}H]SCH23390 and after 15 minutes for [^{3}H]spiperone when incubated at 37° C The specific binding of [^{3}H]SCH23390 showed a linear relationship with protein concentration up to 3.0mg/ml whereas the specific binding of [^{3}H]spiperone increased linearly only up to protein concentrations of 0.18mg/ml Cis-flupenthixol (1μ M) was shown to be a selective displacer for nonspecific binding of [^{3}H]SCH23390 and this ligand was also displaced with high affinity by butaclamol but not by the DA D2 selective drug, sulpiride. Sulpiride (10μ M) was demonstrated to be a selective displacer for nonspecific binding of [^{3}H]spiperone. Drugs active at both DA D1 and D2 receptors, butaclamol and cis-flupenthixol, also displaced [^{3}H]spiperone with high affinity, and there was evidence that the 5-HT_2 antagonist, ketanserin also displaced this ligand Under the present conditions, therefore, [^{3}H]SCH23390 and [^{3}H]spiperone have been considered to be suitable ligands for the investigation of DA D1 and D2 receptors in rat nucleus accumbens.

CHAPTER 7

DISTRIBUTION OF DOPAMINE RECEPTORS IN THE NUCLEUS ACCUMBENS

7.1 INTRODUCTION

There has been histological evidence that the nucleus accumbens is not a homogeneously organized structure (Chronister et al., 1980; Herkenham et al., 1984, de France et al., 1983) and it has been shown in Chapter 4 that the monoamines DA, NA and 5-HT and the metabolites DOPAC, HVA and 5-HIAA have different regional distributions in the nucleus accumbens. Dopamine levels were found to be low in the rostral part of the nucleus accumbens, being significantly lower in the ventrorostral area than in the dorsorostral area, and high in both medial and caudal areas. DA receptors have been classified into two major subtypes (Kebabian and Calne, 1979; Stoof and Kebabian, 1981). DA D1 receptors which are positively coupled to adenylate cyclase and DA D2 receptors which are negatively coupled or uncoupled to this enzyme. Both of these receptor subtypes have been demonstrated to be present in the nucleus accumbens (Savasta et al., 1986, Camus et al., 1986, White and Wang, 1986). The aim of this study was therefore to investigate the distribution of DA D1 and D2 receptors within the nucleus accumbens and to determine whether their distribution correlated with DA content. The functional activity of DA D2 receptors within the nucleus accumbens was also investigated using an in vitro superfusion technique in which DA D2 receptor-mediated inhibition of [^{3}H]DA and [^{14}C]ACh release from prelabelled nucleus accumbens slices was determined. The difference between the effects of the DA D2 agonist, quinpirole and the DA D2 antagonist, sulpiride on [^{3}H]DA and [^{14}C]ACh release was determined and used as a measure of full DA D2 agonist activity. Uptake of [^{3}H]DA and [^{14}C]choline was determined to obtain a measure of the distribution of DA and ACh terminals in different areas of the nucleus accumbens.

7.2 METHODS

7.2.1 Receptor studies

7.2.1.1 Tissue preparation

Male Wistar rats (250-280g) were sacrificed by decapitation and the brains rapidly removed, chilled on ice and then sliced coronally with a McIlwain tissue chopper. The nucleus accumbens was dissected into six areas as described in Section 3.2.1. The dorsomedial area of the nucleus accumbens was further subdivided into medial and lateral regions and the tissue stored at -100° C until assayed. Tissue was pooled from 4 rats for DA D1 receptor assays and from 10 rats for DA D2 receptor assays except for assays of the ventrorostral area, and the medial and lateral parts of the dorsomedial area, when tissue was pooled from 15 rats for the DA D2 receptor assay. The pooled tissue was thawed and homogenized in 40 volumes of ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and 1µM pargyline (pH 7.4 at 25° C) and further prepared as described in Section 3.2.5

7.2.1.2 DA D1 receptor assay

Four hundred microlitres of tissue homogenate was pipetted, in duplicate, into polypropylene incubation tubes and 20µl of [^{3}H]SCH23390 was added, resulting in concentrations ranging from 0.02-3.0 nM in the final incubation mixture. To define nonspecific binding, 10µl of cis-flupenthixol was added to one set of tubes to give a concentration of 1µM in the final incubation mixture. To prevent [^{3}H]SCH23390 binding to 5-HT_2 binding sites 30nM ketanserin was included throughout. The assay followed the method described for DA D1 receptor binding in Section 3.2.5

7.2.1.3 DA D2 receptor assay

Four hundred microlitres of tissue homogenate was pipetted, in duplicate, into polypropylene incubation tubes and 20µl of [^{3}H]spiperone was added, resulting in concentrations ranging from 0.04-2.0 nM in the final incubation mixture. To define nonspecific binding 10µl of sulpiride was added to one set of tubes to give a concentration of 10µ M in the final incubation mixture. To prevent [^{3}H]spiperone binding to 5-HT_2 binding sites, 3nM ketanserin was added throughout. The assay followed the method for DA D2 receptor binding described in Section 3.2.5.

7.2.2 Uptake and release studies

The uptake studies were performed by Dr. V.A. Russell and release studies were performed by Mrs. M.C.L. Lamm.

7.2.2.1 Tissue preparation

Male Wistar rats (250-280g) were decapitated, the brains rapidly removed and sliced coronally as described in Section 3.2.1. The nucleus accumbens was dissected out of the three slices containing this area. In certain experiments dorsal and ventral regions were dissected out of the rostral and medial nucleus accumbens slices. Subsequently the three or four areas were further sliced (0.3 x 0.3 mm) with the McIlwain tissue chopper. These slices were suspended in 250 volumes of Krebs buffer (124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 1.2 mM $CaCl_2$, 26 mM $NaHCO_3$ and 10 mM glucose adjusted to pH 7.3-7.4 by aeration with 95% 02/5% CO_2). Ascorbate (5.7 mM) and nialamide (25µ M) were included to reduce degradation of [^{3}H]DA. Desipramine (10µ M) was added to prevent uptake of [^{3}H]DA into noradrenergic terminals (Hetey and Zimmermann, 1986).

7.2.2.2 Uptake studies

For uptake studies portions of the slice preparation were allowed to equilibrate at 37° C (and 0° C for blanks) for 10 min. The reaction was started by the addition of 0.1µ M [^{3}H]DA (40 Ci/mmol) and 4µ M [methyl^{14}C]choline chloride (52 mCi/mmol). After 15 minutes incubation at 37° C (or 0° C) under an atmosphere of 95% O_2 /5% CO_2 , the preparation was cooled on ice and transferred to ice-cold saline The slices were filtered on GF/C glass fiber filters and washed four times with ice-cold saline The tissue was dissolved in 1M NaOH, neutralized with 1M HCl and Instagel (Packard) added for liquid scintillation counting. Specific [^{3}H]DA and [^{14}C]choline uptake was defined as the difference between the radioactivity taken up by the slices at 37° C and 0° C.

7.2.2.3 Release studies

For release studies (Nurse et al., 1987; Allin et al , 1988) tissue slices (0.3 x 0.3mm) were suspended in 50 volumes of Krebs buffer and similarly loaded with [^{3}H]DA and [^{14}C]choline prior to being transferred to superfusion chambers (6 mg tissue per chamber). The slices were perfused at 37° C with Krebs buffer (0.25 ml/minute). To test the effect of quinpirole and sulpiride, 1-2 five minute fractions were collected to establish baseline release then the chamber inlets were switched to Krebs buffer containing the drug to be tested and 15 minutes later the slices were exposed to Krebs buffer containing 25mM K^+ (isomolar replacement of NaCl by KCl) to stimulate the release of [^{3}H]DA and [^{14}C]ACh. The radioactivity in the eluate fractions and in the tissue slices at the end of the experiment was determined. In order to correct for differences in the amount of tissue in each superfusion chamber, the data were expressed as fractional release (de Langen et al., 1979). The area of the 25 mM K^+-induced peak of fractional release of radioactivity was used as a measure of neurotransmitter release

7.3 RESULTS

7.3.1 Distribution of DA D1 receptors in the nucleus accumbens

Receptor density (Bmax) and affinity (K_D) values for DA D1 and D2 receptors in the six areas of the nucleus accumbens are shown in Table 7.1. The DA D1 receptor number was significantly lower in the rostral area than in the medial and caudal areas (Fig. 7.1). There were no dorsoventral differences. The K_D value for DA D1 receptors in the nucleus accumbens was significantly lower in the dorsorostral area than in any other area.

7.3.2 Distribution of DA D2 receptors in the nucleus accumbens

The DA D2 receptor number was significantly lower in the ventrorostral area, and significantly higher in the dorsomedial area than in any other area. The receptor density in the dorsorostral, ventromedial, dorsocaudal and ventrocaudal areas was similar (Table 7.1, Fig. 7.1). The K_D value for [^{3}H]spiperone was significantly lower in the ventrorostral area than in any other area. There were no significant differences observed in Bmax or K_D between medial and lateral regions of the dorsomedial area of the nucleus accumbens (Table 7.2).

7.3.3 Uptake of [^{3}H]DA and [^{14}C]choline in different areas of the nucleus accumbens

[^{3}H]DA uptake into the rostral nucleus accumbens slice preparation was significantly lower than in the medial slice preparation (Table 7.3). [^{3}H]DA uptake in the ventrorostral area was significantly lower than in the dorsorostral area (Table 7.4). There was no significant difference in [^{3}H]DA uptake between dorso- and ventromedial areas of the nucleus accumbens (Table 7.4). The uptake of [^{14}C]choline in the medial nucleus accumbens slice was significantly greater than in the rostral (Tables 7.3 and 7.4) and caudal slices (Table 7.3). Uptake of [^{14}C]choline was significantly lower in the ventrorostral than in the dorsorostral

TABLE 7.1

Distribution of DA D1 and D2 receptor density (Bmax) and affinity (K_D) in the nucleus accumbens of the rat.

	[^{3}H]SCH23390 binding		[^{3}H]SPIPERONE binding	
Nucleus accumbens area	Bmax (fmol/mg protein)	K_D (nM)	Bmax (fmol/mg protein)	K_D (nM)
dorsorostral	124.7± 18.9[a]	0.125± 0.028[b]	101.1± 7.1	0.054± 0.013
ventrorostral	137.1± 21.0[a]	0.294± 0.041	15.3± 1.1[c]	0.024± 0.013[c]
dorsomedial	593.8± 45.4	0.377± 0.026	195.8± 10.4[d]	0.054± 0.005
ventromedial	498.6± 40.8	0.360± 0.026	104.1± 5.7	0.041± 0.003
dorsocaudal	706.8± 82.2	0.364± 0.052	102.8± 6.2	0.049± 0.007
ventrocaudal	681.6± 104.8	0.419± 0.067	116.3± 4.4	0.053± 0.009

Results are the mean ± SEM of 5-6 observations.
For DA D1 receptors a two way ANOVA was fitted to both variables (Bmax and K_D) with RAT and AREA as factors. Both showed significant differences ($P = 0.0016$ and $P = 0.0001$ respectively). As the variance of the observations increased with their mean, log transformation was applied to the data. Using the transformed data the areas were compared using Tukey's studentized range test shown in Appendix C 2. For DA D2 receptors a two way ANOVA was also fitted. RAT was not significant ($P = 0.55$) and was therefore dropped from the model. Areas were then compared using Tukey's studentized range test shown in Appendix C 2.

Significant difference[a] vs dorsomedial, ventromedial, dorsocaudal and ventrocaudal areas;
[b] vs ventrorostral, dorsomedial, ventromedial, dorsocaudal, and ventrocaudal areas;
[c] vs dorsorostral, dorsomedial, ventromedial, dorsocaudal, and ventrocaudal areas;
[d] vs dorsorostral, ventrorostral, ventromedial, dorsocaudal, and ventrocaudal areas ($P < 0.05$).

area of the nucleus accumbens (Table 7.4). There were no significant dorsoventral differences in or [^{14}C]choline uptake in the medial slice.

7.3.4 Release of [^{3}H]DA and [^{14}C]ACh in different areas of the nucleus accumbens

The K^+-stimulated release of [^{3}H]DA was not significantly different in rostral, medial and caudal nucleus accumbens slices (Table 7.3). The K^+-stimulated release of [^{3}H]DA was significantly greater in the dorsorostral than in the ventrorostral area and in the dorsomedial

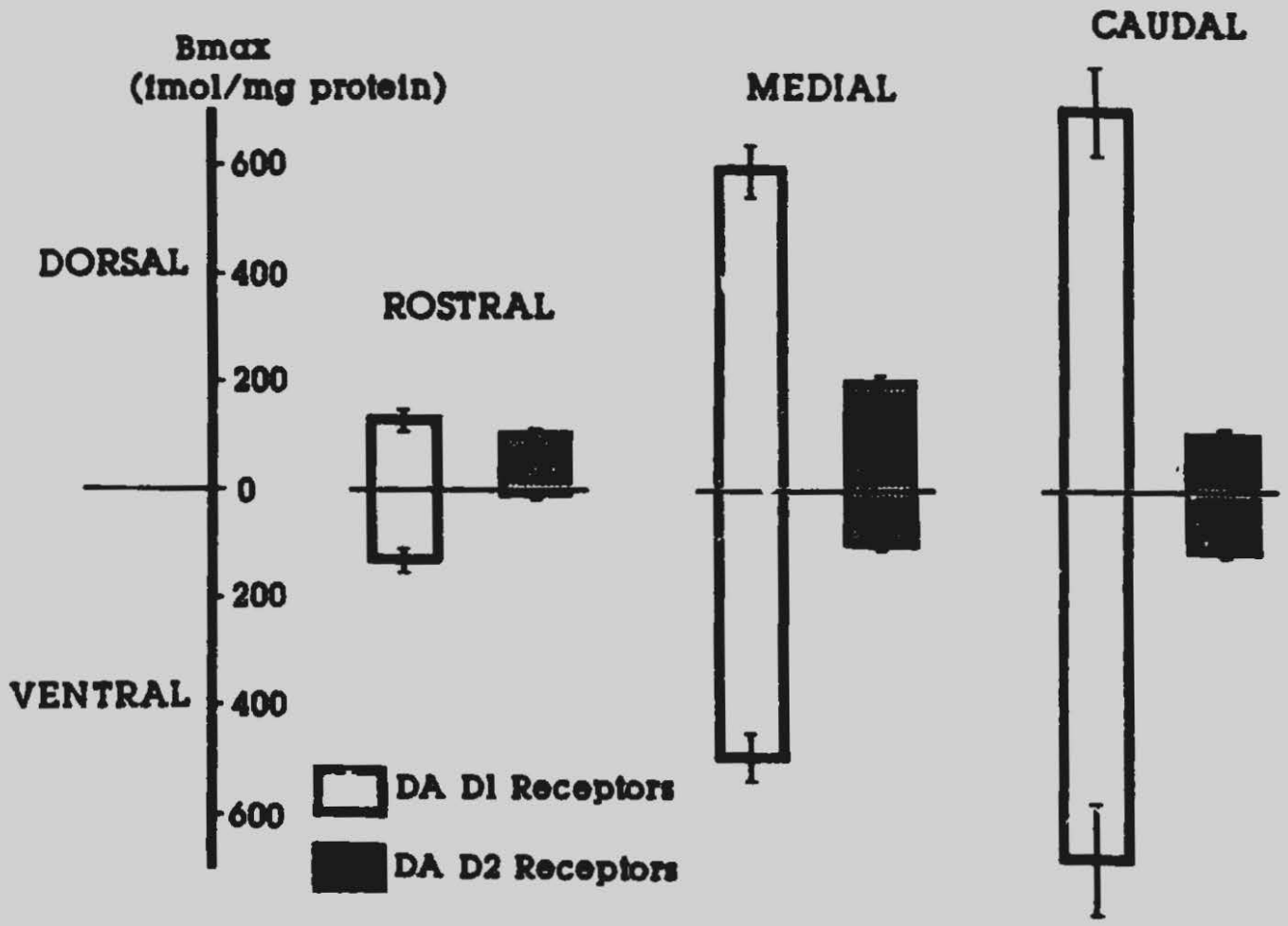

Fig. 7.1 Distribution of DA D1 and D2 receptors in the nucleus accumbens of the rat. The mean ± SEM of 6-7 observations are shown.

TABLE 7.2

Dopamine D2 receptor density (Bmax) and affinity (K_D) in the dorsomedial area of the rat nucleus accumbens.

	[^{3}H]SPIPERONE binding	
Nucleus accumbens area	Bmax (fmol/mg protein)	K_D (nM)
medial	179 7± 4 6	0 039± 0.007
lateral	177 5± 10 2	0.038± 0 007

Results are the mean± SEM of 3 observations
Student's t-test showed no significant differences.

than in the ventromedial area of the nucleus accumbens (Table 7 5) The K^+-stimulated release of [^{14}C]ACh in the medial nucleus accumbens slice preparation was significantly greater than in the rostral and caudal slices (Table 7 3) K^+-stimulated release of [^{14}C]ACh was significantly higher in the dorsorostral than in the ventrorostral area but there were no differences observed in [^{14}C]ACh release between dorsomedial and ventromedial areas of the nucleus accumbens (Table 7 5) No significant DA D2 receptor-mediated inhibition of K^+-stimulated release of [^{3}H]DA or [^{14}C]ACh was observed in the ventrorostral area of the nucleus accumbens (Table 7 5, Fig 7 2 In the dorsomedial area of the nucleus accumbens the DA D2 receptor mediated inhibition of K^+ stimulated release of [^{14}C]ACh was significantly greater than in the dorsorostral, ventrorostral and ventromedial areas (Table 7.5, Fig. 7 2)

7.4 DISCUSSION

The presence of both DA D1 and D2 receptors in the nucleus accumbens was demonstrated in this investigation The DA D1 receptor density was 3-9 times higher than the DA D2 receptor density in the nucleus accumbens This is consistent with the findings of Hyttel and Arnt, (1986), who reported that the DA D1 receptor density was 2-6 times higher than the D. D2 receptor density in rat striatum and that the ratio of D1/D2 receptors increased from the

TABLE 7.3

Uptake of [^{3}H]DA and [^{14}C]choline and the K^+-stimulated release of [^{3}H]DA and [^{14}C]ACh in different areas of the rat nucleus accumbens

	Nucleus accumbens area		
Neurotransmitter, measure	Rostral	Medial	Caudal
DA,			
[^{3}H]DA uptake (pmol/mg prot/15min)	9 2± 0 92	15 7± 0 92[a]	13 4± 0 66
K^+-evoked release of [^{3}H]DA	0 154± 0.007	0.148± 0 006	0 142± 0 003
ACh,			
[^{14}C]choline uptake (pmol/mg prot/15min)	360± 12	548± 18[b,c]	390± 15
K^+-evoked release of [^{14}C]ACh	0.112± 0 003	0 215± 0 006	0 191± 0 003[c]

Neurotransmitter uptake and release studies were performed by Dr V A Russell and Mrs M.C L Lamm Results are the mean± SEM of 4 to 12 observations. The data for [^{3}H]DA uptake were analysed using paired t-tests To reach Bonferroni significance the P-value must be < 0 016 The data for [^{3}H]DA and [^{14}C]ACh release were analysed using ANOVA (AREA was not significant for [^{3}H]DA release, P = 0 18, but was significant for [^{14}C]ACh release, P = 0 0001). Simultaneous confidence intervals based on Tukey's studentized range test were performed (Ryan-Einot Gabriel-Welsch procedure) shown in Appendix C 3 Since the standard deviations for [^{14}C]ACh uptake data for the 3 slices were nearly the same, the data were analysed using ANOVA (SLICE was significant, P = 0 0045) followed by Tukey's studentized range test shown in Appendix C 3

[a] Significant difference vs rostral slice, P < 0 016, [c] P < 0 05; [b] vs caudal slice , P < 0 05

striatum via the nucleus accumbens to the olfactory tubercle The present study also provides evidence for the heterogeneity of distribution of both DA D1 and D2 receptors within the nucleus accumbens of the rat.

The distribution of DA D1 receptors in the nucleus accumbens was similar to the DA content, being low in the rostral and high in the medial and caudal areas of the nucleus accumbens DA uptake also increased from the rostral to the medial part of the nucleus accumbens, indicating that dopaminergic innervation is significantly lower in the rostral than in the medial nucleus accumbens However, there were no dorsoventral differences observed in DA D1 receptor density in any coronal section of the nucleus accumbens whereas in the rostral nucleus

TABLE 7.4

Uptake of [^{3}H]DA and [^{14}C]choline in different areas of the rat nucleus accumbens

Nucleus accumbens area	[^{3}H]DA UPTAKE (pmol/mg protein/15min)	[^{14}C]CHOLINE UPTAKE (pmol/mg protein/15min)
dorsorostral	5.59± 0.75	277± 27[b]
ventrorostral	2.79± 0.17[a]	204± 11[a]
dorsomedial	7.88± 0 80	360± 23
ventromedial	8.10± 0 56	376± 16

Neurotransmitter uptake studies were performed by Dr V.A. Russell and Mrs. M.C.L. Lamm Results are the mean± SEM of 5 observations. To stabilize the variance the log transformed data were analysed. A two way ANOVA model was fitted with SLICE (rostral/medial) and AREA (ventral/dorsal) as factors SLICE, AREA and the SLICE*AREA interaction were significant (P=0.001, 0 0119 and 0.0055 respectively) The AREA*SLICE interaction data was compared using Tukey's studentized range test shown in Appendix C 4.
Significant difference[a] vs dorsorostral, dorsomedial, and ventromedial areas, [b] vs dorsomedial and ventromedial areas, ($p<0.05$)

accumbens the DA concentration was 2.5 times lower ventrally than dorsally and uptake was twice as low in the ventral than in the dorsal area It therefore appears that although the ventrorostral area of the nucleus accumbens is more sparsely innervated by dopaminergic neurons than the dorsorostral area, similar densities of post synaptic DA D1 receptors are present DA turnover has been shown to be significantly higher in the ventrorostral area of the nucleus accumbens (Section 4.3 2) than in the dorsorostral area suggesting that although there are fewer DA terminals in the ventrorostral area, these neurons are more active than those in the dorsorostral area and can activate a similar number of postsynaptic DA D1 receptors.

The distribution of DA D1 receptors in the striatum has also been shown to have a similar distribution to DA content (Section 4 3 3) in that it has a decreasing rostrocaudal gradient and a decreasing lateral to medial gradient without any apparent gradient along the dorsoventral axis (Savasta et al., 1986) although other authors have reported a more uniform DA D1 receptor distribution (Altar and Hauser, 1987).

The K_D value for DA D1 receptors in the nucleus accumbens was significantly lower in the

TABLE 7.5

K^+-stimulated release of [^{3}H]DA and [^{14}C]ACh from different areas of the rat nucleus accumbens.

Neurotransmitter, Nucleus accumbens area	K^+-stimulated release			
	Addition to the superfusion buffer			
	Nil	Quinpirole (1 µ M)	Sulpiride (1 µ M)	Sulpi Quir
[^{3}H]DA				
dorso rostral	0.156± 0.0058	0.127± 0.0045[a,b]	0.177± 0.0072[a]	0.050
ventro rostral	0.126± 0 0097[c]	0.110± 0.0077[a,b,c]	0.126± 0 0100[c]	0 016[c,d,e,g]
dorso medial	0 166± 0 0067	0.142± 0.0040[a,b]	0.195± 0.0052[a]	0.053
ventro medial	0.141± 0 0035[d]	0.115± 0 0070[a,b,d]	0.158± 0 0076[d]	0 043
[^{14}C]ACh				
dorso rostral	0.127± 0 0103[d,e]	0.098± 0 0051[a,b,d,e]	0.137± 0.0111[d,e]	0.039
ventro rostral	0.105± 0 0094[c,d,e]	0.092± 0 0057[a,b,d,e]	0.101± 0.0071[c,d,e]	0.009[c,d,e,g]
dorso medial	0.233± 0 0065	0.174± 0.0047[a,b]	0.239± 0 0069	0.065[c,e,f]
ventro medial	0.222± 0 0062[d]	0.190± 0.0045[a,b]	0.227± 0 0073[d]	0 037

Neurotransmitter release studies were performed by Mrs. M.C.L. Lamm.
Results are the mean± SEM of 10 observations. A three way ANOVA model with factors SLICE (rostral/medial), AREA (ventral/dorsal) and TREATMENT (nil/quinpirole/sulpiride) was fitted For [^{3}H]DA release SLICE, AREA and TREATMENT were significant as well as the AREA*TREATMENT interaction (P=0.0001, 0.0001, 0 0001 and 0.0012 respectively). Tukey's studentized range test was applied to compare the 6 means of the AREA*TREATMENT interaction, shown in Appendix C.5. For [^{14}C]ACh release ANOVA indicated all effects to be significnt (P<0.005) except the AREA*SLICE*TREATMENT interaction (P=0.640). Tukey's studentized range test was used to compare the means of the SLICE*AREA interaction, the SLICE*TREATMENT interaction and the AREA*TREATMENT interaction, shown in Appendix C.5 Pairwise differences between quinpirole and sulpiride data were formed and analysed for AREA and SLICE differences using ANOVA. This interaction was not significant for [^{3}H]DA or [^{14}C]ACh release (P=0.073 and 0.878 respectively) This was followed by Tukey's studentized range test shown in Appendix C.5.
Significant difference (p<0 05); [a] vs control; [b] vs sulpiride, [c] vs dorsorostral, [d] vs dorsomedial; [e] vs ventromedial; [f] vs ventrorostral, [g] Not significantly different from zero.

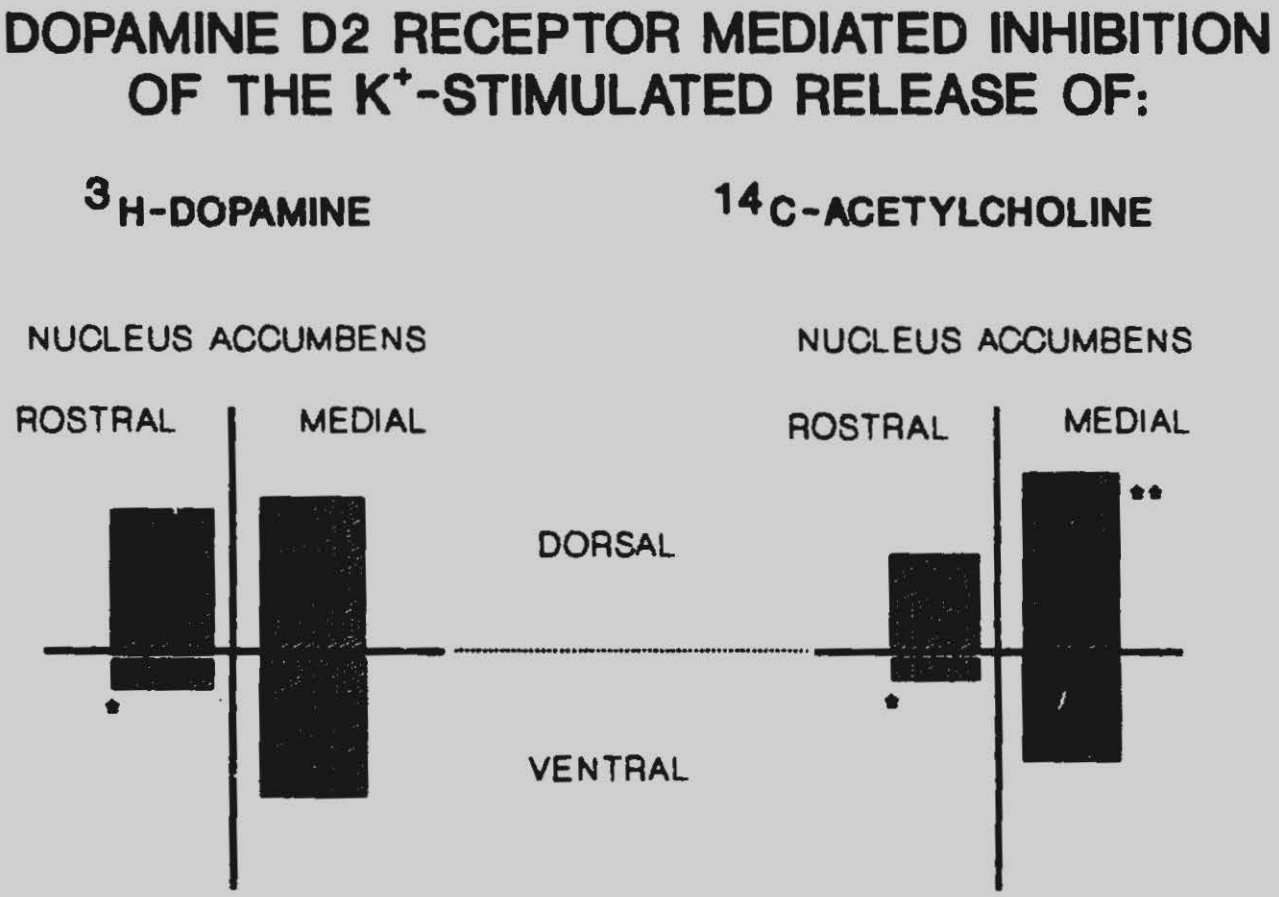

Fig. 7.2 Diagrammatic representation of DA D2 receptor mediated inhibition of the K^+-stimulated release of [^{3}H]DA and [^{14}C]ACh in the nucleus accumbens of the rat.

dorsorostral area than in any other area. This could be due to reduced accuracy in measurement of K_D when the receptor number is low. It can be seen from Table 7.1 that the DA D1 receptor K_D in the ventrorostral area was also lower than that measured in the medial and caudal areas.

In contrast to DA D1 receptors, the DA D2 receptor distribution in the nucleus accumbens does not follow DA terminal distribution as measured by either content (Section 4 3.1) or uptake (Table 7 4). The DA D2 receptor density was significantly lower in the ventrorostral and higher in the dorsomedial areas than in any other area of the nucleus accumbens. There was no difference in DA D2 receptor density in medial and lateral parts of the dorsomedial area, thus localizing the highest DA D2 receptor density in the nucleus accumbens to this area. The DA D2 receptor density in the dorsorostral, ventromedial, dorsocaudal and ventrocaudal areas was similar whereas this was not the case for the DA content and uptake. The DA concentration was 2.4 to 3 5 times lower in the dorsorostral nucleus accumbens than in the ventromedial, dorsocaudal and ventrocaudal areas. DA uptake in the dorsorostral nucleus accumbens was not significantly lower than in the ventromedial area. DA uptake was found to be similar in dorsorostral, ventromedial and dorsomedial areas. A marked difference was observed in the ventrorostral area of the nucleus accumbens where DA innervation was half that observed in the dorsorostral area while the DA D2 receptor density was barely detectable. This would imply that the DA terminals in the ventrorostral area of the nucleus accumbens have considerably fewer inhibitory DA D2 autoreceptors than the DA terminals in the dorsorostral, medial and caudal areas. This could account for the greater activity of the DA terminals in the ventrorostral area. The absence of DA autoreceptor-mediated feedback inhibition of DA release from these terminals could give rise to greater DA release, depletion of DA stores and the relatively greater DA turnover observed in this area (Section 4 3 2). In agreement with there being very few DA D2 receptors in the ventrorostral area of the nucleus accumbens, no significant DA D2 receptor mediated inhibition of the K^+-stimulated release of [^{3}H]DA or [^{3}H]ACh was observed in this area (Table 7 5), which confirms the suggestion that DA release -

from DA terminals in the ventrorostral area of the nucleus accumbens is not regulated by DA autoreceptor inhibition. The lack of synthesis modulating DA autoreceptors in the frontal cortex has also been suggested (Galloway et al., 1986, Kilts et al., 1987) A marked difference between DA D2 receptor density and DA innervation was also observed in the dorsomedial area of the nucleus accumbens. Twice as many DA D2 receptors were present in the dorsomedial than in the ventromedial and dorsorostral areas, whereas the proportion of DA terminals was similar in these areas The increased number of DA D2 receptors in this area is therefore not due to the presence of a greater number of terminals. However there could be more DA D2 autoreceptors on the dopaminergic terminals or DA D2 receptors may be situated postsynaptically, for example on cholinergic terminals The former explanation does not seem likely as DA turnover was not lower in the dorsomedial area (Section 4.3 2) and DA autoreceptor function was not increased in the dorsomedial area The suggestion that there may be more DA D2 receptors postsynaptically on cholinergic neurons was confirmed by the [^{14}C]ACh release data The DA D2 receptor-mediated inhibition of the K^+-stimulated release of [^{14}C]ACh from dorsomedial nucleus accumbens slices was 1 7 times greater than in the dorsorostral and ventromedial areas This is consistent with there being 1 9 times as many DA D2 receptors in the dorsomedial area The data strongly suggests that there is greater DA D2 receptor-mediated inhibitory regulation of ACh release in the dorsomedial area than other areas of the nucleus accumbens The increased activity of cholinergic neurons observed in the medial nucleus accumbens is consistent with the results of Jones et al., (1981) who reported a greater increase in locomotion after injection of dopamine into the medial than into any other area of the nucleus accumbens. There were no dorsoventral differences in [^{14}C]choline uptake or [^{14}C]ACh release in the medial area of the nucleus accumbens to account for the greater DA D2 receptor function. It would therefore appear that there are more DA D2 receptors on ACh terminals in the dorsomedial than in any other area of the nucleus accumbens

The distribution of DA D2 receptors in the striatum has been reported to show some overlap with areas of high DA content (Section 4 3 3) and DA D1 receptor localization (Altar and

Hauser, 1987; Joyce and Marshall, 1987) but the distributions of DA D1 and D2 receptors do not appear to be entirely superimposable as DA D2 receptors have been shown to be highly concentrated in the dorsolateral part of the rostral striatum (Joyce and Marshall, 1987) whereas rostrally DA levels were found to be higher medially (Section 4.3.3). A high density of DA D2 receptors has also been reported to be present midway along the rostrocaudal axis of the striatum (Camus et al., 1986) whereas DA levels have been shown to be highest in the rostral part of the striatum (Section 4.3.3; Beal and Martin, 1985). However in the globus pallidus, where the lowest concentration of DA was measured (Section 4.3.3), and where very low DA uptake has been demonstrated (Hardy et al., 1987), densities of DA D1 and D2 receptors have also been reported to be lower than in other parts of the striatum (Dubois et al., 1986).

7.5 SUMMARY

The distribution of DA D1 and D2 receptors in the nucleus accumbens was not superimposable although there was some overlap. DA D1 receptor density roughly followed dopaminergic innervation being low in the rostral and high in the medial and caudal areas. However in the rostral part of the nucleus accumbens there was very sparse dopaminergic innervation in the ventrorostral area while DA D1 receptors were evenly distributed dorsoventrally. The highest DA turnover was observed in the ventrorostral area and this suggests that the relatively few, more active DA terminals can activate a similar number of postsynaptic DA D1 receptors as in the dorsorostral area. The distribution of DA D2 receptors did not follow the DA uptake or DA content in the nucleus accumbens. DA D2 receptor density was similar in dorsorostral, ventromedial and caudal areas. In the ventrorostral area dopaminergic innervation was half of that observed in the dorsorostral area but the DA D2 receptor density was barely detectable. It appears, therefore, that there are very few inhibitory DA D2 autoreceptors in this area. In the dorsomedial area of the nucleus accumbens the increased number of DA D2 receptors does not appear to be due to an increased number of dopaminergic terminals, or to the presence of more

DA D2 autoreceptors on DA terminals in this area However, there is evidence for a greater concentration of postsynaptic DA D2 receptors on ACh terminals in the dorsomedial area than in other areas of the nucleus accumbens

CHAPTER 8

EFFECT OF DESIPRAMINE TREATMENT ON DOPAMINE D1 AND D2 RECEPTORS IN THE NUCLEUS ACCUMBENS

8.1 INTRODUCTION

It has been suggested that a reward process in which the mesolimbic dopaminergic system is involved is hypoactive in depression (Fibiger and Phillips, 1981, Willner, 1983, Section 2.4) Antidepressants have been shown to affect noradrenergic and serotonergic systems, and there has also been evidence that chronic t. ·ment with antidepressants leads to an increase in dopamine transmission, particularly in the mesolimbic system (Spyraki et al., 1982, Willner, 1983, Section 2.3) This has been suggested to be due to supersensitivity of the postsynaptic DA receptors (Spyraki and Fibiger, 1981) DA receptors have been divided into two major subtypes (Kebabian and Calne, 1979, Stoof and Kebabian, 1981) Dopamine D1 receptors are positively coupled to adenylate cyclase and dopamine D2 receptors are negatively coupled or uncoupled to this enzyme Several authors have reported that chronic treatment with antidepressants causes no change in [^{3}H]spiperone binding in the striatum or forebrain of the rat (Rehavi et al., 1980, Peroutka and Snyder, 1980, Klimek and Nielsen, 1987) while in one study [^{3}H]DA binding was found to be decreased in the striatum (Lee and Tang, 1982). In a recent study Klimek and Nielsen, (1987) have reported decreased DA D1 receptor binding in rat striatal and limbic areas

The aim of the present study was therefore to investigate the effect of chronic treatment with the antidepressant drug, desipramine, on DA D1 and D2 receptor binding in the nucleus accumbens

3.2 METHODS

3.2.1 Animals

Male Wistar rats (170-210g at the beginning of the experiment) were housed in groups of 5 per cage under standard laboratory conditions as described in Section 3.1.1. Test groups received the antidepressant drug, desipramine, (20mg/kg/day) in their drinking water for 14 or 28 days. The amount of desipramine dissolved in the water was adjusted according to the average weight and water intake of each group of rats so that the average dose remained as close to 20mg/kg/day as possible. The concentration was approximately 10mg desipramine/100ml water.

3.2.2 Tissue preparation

Rats (250-280g) were sacrificed by decapitation and the brains rapidly removed, chilled on ice and then sliced coronally with a McIlwain tissue chopper. The nucleus accumbens was dissected out of the three brain slices which contained this area, as described in Section 3.2.1., and the whole nucleus accumbens was stored in liquid nitrogen until assayed. In the case of rats treated for 14 days, tissue from individual rats was used for DA D1 receptor assays. In the case of rats treated for 28 days, binding assays for both DA D1 and D2 receptors were performed on tissue pooled from 3 rats. The tissue was thawed and homogenized in 40 volumes of ice cold 50mM Tris HCl buffer containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic acid and 1µ M pargyline (pH 7.4 at 25° C) and further prepared as described in Section 3.2.5.

3.2.3 DA D1 receptor assay

Four hundred microlitres of tissue homogenate was pipetted, in duplicate, into polypropylene incubation tubes and 20µl of [^{3}H]SCH23390 was added, resulting in concentrations ranging from 0.02-3.0nM in the final incubation mixture. To define nonspecific binding, 10µl of cis-

flupenthixol was added to one set of tubes to give a concentration of 1µ M in the final incubation mixture. To prevent [^{3}H]SCH23390 binding to 5-HT_2 binding sites 30nM ketanserin was included throughout. The assay followed the method described for DA D1 receptor binding in Section 3.2.5. Bmax and K_D values were obtained using the nonlinear least squares curve-fitting program LIGAND as described in Section 3.2.6.

8.2.4 DA D2 receptor assay

Four hundred microlitres of tissue homogenate was pipetted, in duplicate, into polypropylene incubation tubes and 10µl of [^{3}H]spiperone was added resulting in concentrations ranging from 0.04-2.0nM in the final incubation mixture. To define nonspecific binding 10µl of sulpiride was added to one set of tubes to give a concentration of 10µ M in the final incubation mixture. To prevent [^{3}H]spiperone binding to 5-HT_2 binding sites, 3nM ketanserin was added throughout. The assay followed the method for DA D2 receptor binding described in Section 3.2.5. Bmax and K_D values were obtained using the nonlinear least squares curve-fitting program LIGAND as described in Section 3.2.6.

8.3 RESULTS

8.3.1 Effect of 14 day treatment on DA D1 receptors in the nucleus accumbens

No significant differences were observed in the DA D1 receptor number (Bmax) or affinity (K_D) between controls and rats treated with desipramine for 14 days (Table 8.1).

8.3.2 Effect of 28 day treatment on DA D1 and D2 receptors in the nucleus accumbens

No significant differences in either the Bmax or K_D were observed for DA D1 receptors between control and treated animals. There were also no differences observed in the Bmax or K_D of DA D2 receptors between control and treated animals (Table 8.2).

TABLE 8.1

Effect of 14 day treatment with desipramine (DMI) on DA D1 receptor density (Bmax) and affinity (K_D) in the nucleus accumbens of the rat.

	[^{3}H]SCH23390 binding	
	Bmax (fmol/mg protein)	K_D (nM)
CONTROL	515.6 ± 51.7	0.366 ± 0.039
DMI	559.1 ± 85.1	0.321 ± 0.045

Results are the mean ± SEM of 5 observations. Student's t-test showed no significant treatment differences.

8.4 DISCUSSION

No significant difference in DA D1 receptor density or affinity was observed in the nucleus accumbens of controls and rats treated with desipramine for either 14 or 28 days. This is in contrast with the findings of Klimek and Nielsen, (1987), which indicated a decrease in the number of [^{3}H]SCH23390 binding sites in limbic tissue after chronic treatment with antidepressant drugs or electroconvulsive shock. The apparent discrepancy could be due to differences in the binding conditions, for example the inclusion of NaCl and the exclusion of the Ca^{++} chelator, EGTA, in the incubation buffer. In the study of Klimek and Nielsen (1987) the drug was administered twice daily as an intraperitoneal injection which could have resulted in stress and accounted for the differences in their finding. They also reported on the limbic system as including the olfactory tubercle, preoptic area, septum, and amygdala as well as the nucleus accumbens.

The lack of effect of desipramine treatment on DA D1 and D2 receptor affinity is in agreement with several previous reports (Klimek and Nielsen, 1987; Rehavi et al., 1980; Peroutka and Snyder, 1980).

Chronic desipramine treatment was not found to affect DA D2 receptor density in the nucleus

TABLE 8.2

Effect of 28 day treatment with desipramine (DMI) on DA D1 and D2 receptor density (Bmax) and affinity (K_D) in the nucleus accumbens of the rat.

	[^{3}H]SCH23390 binding		[^{3}H]SPIPERONE binding	
	Bmax (fmol/mg protein)	K_D (nM)	Bmax (fmol/mg protein)	K_D (nM)
CONTROL	580.6± 34.5	0.405± 0.036	124.9± 10.0	0.064± 0.007
DMI	555.4± 49.3	0.377± 0.042	129.5± 7.6	0.070± 0.006

Results are the mean± SEM of 6 observations. Student's t-test showed no significant treatment differences.

accumbens. This is in agreement with previous reports on DA D2 receptor binding in striatum (Peroutka and Snyder, 1980) and forebrain (Rehavi et al., 1980) tissue after chronic antidepressant treatment. Also in support of the lack of DA D2 receptor changes following antidepressant treatment the dose dependent quinpirole-mediated inhibition of the electrically stimulated release of [^{3}H]DA and [^{14}C]ACh from nucleus accumbens slices was not found to be influenced by chronic antidepressant treatment (Reyneke, et al., 1989). However these results are not in agreement with the suggestion that postsynaptic DA receptors become supersensitive following antidepressant treatment which was based on the observation that apomorphine-induced locomotor activity was increased in antidepressant treated animals (Spyraki and Fibiger, 1981).

It has been reported, using biochemical (Serra et al., 1979) and electrophysiological (Chiodo and Antelman, 1980) methods, that chronic antidepressant treatment causes DA autoreceptor subsensitivity. This has been suggested since chronic antidepressant treatment was shown to reverse the effect of small doses of apomorphine on motor activity and DA synthesis in the striatum (Serra et al., 1979), and repeated antidepressant treatment progressively attenuated the depression by apomorphine of the spontaneous activity of single DA cells (Chiodo and Antelman, 1980). However, in contrast to these reports, Holcomb et al., (1982) proposed that

striatal DA autoreceptors are not influenced by chronic antidepressant treatment as the apomorphine induced reversal of dihdroxyphenylalanine (DOPA) elevation after gamma-butyrolactone administration was similar in treated and control animals. Waldmeier, (1984) also provided biochemical evidence for lack of DA autoreceptor subsensitivity after chronic antidepressant treatment.

The antidepressant induced increase in dopaminergic activity observed in behavioural studies was therefore not due to a direct effect on either DA D1 or D2 receptors. However changes could possibly have occurred in second messenger systems. It is known that DA D1 receptor stimulation increases cAMP formation and that DA D2 receptor stimulation either decreases or has no effect on cAMP production (Stoof et al., 1987, Karobath, 1975). Antidepressants have been shown to inhibit DA sensitive adenylate cyclase activity in cell free striatal homogenates and chronic desipramine treatment was reported to reduce cAMP-dependent protein kinase activity (Moyer et al., 1986). DA D2 receptor activation has also been reported to inhibit inositol-phosphate turnover (Fisher and Agranoff, 1987). Antidepressants also interact with some inositide-linked receptors (Fisher and Agranoff, 1987).

It has also been suggested that the effects of antidepressants on DA transmission may be an indirect consequence of their interactions with noradrenergic or serotonin systems (Waldmeier, 1984). Green and Deakin, (1980) found that electroconvulsive therapy-induced enhancement of the increase in locomotor activity caused by apomorphine depended on the integrity of the noradrenergic system, and Nurse et al., (1985) reported that chronic treatment with desipramine attenuated α-adrenoceptor-mediated inhibition of the K^+-evoked release of [^{3}H]DA from nucleus accumbens slices.

These results indicate, therefore, that the effects of chronic antidepressant treatment on the nucleus accumbens cannot be attributed to changes in either DA D1 or D2 receptor binding.

8.5 SUMMARY

The binding of [^{3}H]SCH23390 to DA D1 receptors was measured in the nucleus accumbens of rats treated with desipramine for 14 days DA D1 and D2 receptor binding using [^{3}H]SCH23390 and [^{3}H]spiperone, respectively, as ligands, was determined in rats treated with desipramine for 28 days Neither Bmax nor K_D values were influenced by chronic desipramine treatment Therefore the results suggest that the therapeutic effect of chronic antidepressant treatment cannot be attributed to changes in either DA D1 or D2 receptor number or affinity

CHAPTER 9

GENERAL SUMMARY AND CONCLUSIONS

Depression has been suggested to be associated with noradrenergic dysregulation, serotonergic hypoactivity, as well as hypoactivity of a reward system in which the mesolimbic dopaminergic neurons are involved (Section 2 4). The nucleus accumbens is a major terminal area for these mesolimbic dopaminergic neurons (Section 1 2 2), and is also known to receive noradrenergic and serotonergic innervation (Sections 1 2 1 and 1 2 3) The nucleus accumbens does not appear to be a homogeneously organized structure and interactions between the neurotransmitter systems have been reported to occur in this area The striatum is a major terminal area for the nigrostriatal dopaminergic neurons (Section 1 2 2) and has been studied as a means of comparison with the nucleus accumbens

The investigation of monoamine and DA receptor distribution in six areas of the nucleus accumbens and of monoamine distribution in eighteen areas of the striatum was enabled by the development of the dissection technique described in Section 3 2 1

In this study the regional distribution of the monoamines in the nucleus accumbens was found to be similar in that lower concentrations were observed in the rostral than in the caudal area DA concentrations were considerably higher than NA and 5 HT concentrations DA and its metabolites DOPAC and HVA were found to be significantly higher in the medial and caudal areas than in the rostral area of the nucleus accumbens of the rat Significant dorsoventral differences were also observed It appears that there are fewer DA terminals rostrally than medially and significantly fewer ventrorostrally than dorsorostrally because of the lower [^{3}H]DA uptake observed in these areas (Section 7 3 3) NA concentrations were found to be significantly higher in the caudal area of the nucleus accumbens than in the rostral and medial areas No dorsoventral differences were observed Appreciable levels of 5-HT and its

metabolite 5-HIAA were found in the nucleus accumbens The concentration of 5-HT was highest in the ventrocaudal, dorsocaudal and ventromedial areas of the nucleus accumbens and a decreasing ventrocaudal/dorsorostral concentration gradient was evident These results substantiate evidence of noradrenergic and serotonergic innervation of the nucleus accumbens and show that there is considerable overlap between the various monoaminergic terminals in the nucleus accumbens in which areas interactions may take place. However there are nevertheless finer differences in distributions of monoamine neurotransmitters in the nucleus accumbens, and any further investigations of this nucleus should take these observations into account

In the nucleus accumbens, the highest DA turnover coincided with the lowest DA content, that is, in the rostral area It has therefore been proposed that MAO activity is higher in the rostral than in the caudal area The 5-HIAA/5 HT ratio was found to be higher in the dorsorostral area than in the ventrocaudal area and this finding supports the proposal of a descending rostrocaudal distribution of MAO activity There were no differences in HVA/DOPAC ratios and this suggests a uniform COMT activity throughout the nucleus accumbens

In the striatum DA levels were found to be significantly higher rostrally than caudally This finding suggests a greater number of dopaminergic terminals in the rostral than the caudal area The globus pallidus was found to contain lower DA concentrations than the striatum, an observation which supports reports of a sparse dopaminergic innervation of this area In the present study DA levels were found to be highest in the two most rostral slices of the striatum The nucleus accumbens contained in these two brain slices also had the highest DA levels It is possible therefore that dopaminergic terminals of neurons projecting from the substantia nigra and VTA of the midbrain to the striatum and nucleus accumbens are concentrated in this area

The presence of NA in the striatum implies that this area is innervated by noradrenergic neurons. However, NA levels were found to be 19 3 to 369 1 times lower than DA concentrations in the striatum and more homogeneously distributed than in the nucleus

accumbens. The globus pallidus has previously been shown to be the only area of the striatum where NA could be detected and therefore the higher levels of NA in the ventromedial part of slice 4 and possibly also the ventromedial part of slice 3 may be due to the presence of the globus pallidus in these areas NA was observed to be concentrated in the ventrocaudal part of the striatum

5-HT and 5-HIAA were found to be significantly higher in the ventral than in the dorsal areas and to be higher in the caudal than in the rostral area of the striatum These findings support evidence for a greater concentration of serotonergic terminals in the ventral than in the dorsal striatum with a slightly greater concentration being found in the caudal part of the striatum

Regional differences in DA turnover were not observed in the striatum, as were found in the nucleus accumbens DOPAC/DA ratios were found to be lower in the striatum than in the nucleus accumbens This implies lower, more uniform MAO activity in the striatum than in the nucleus accumbens. HVA/DOPAC ratios were found to be higher in the striatum than the nucleus accumbens, suggesting higher COMT activity in the striatum than in the nucleus accumbens 5-HIAA/5-HT ratios appeared to be slightly higher in the striatum than in the nucleus accumbens The 5-HIAA/5-HT ratio and therefore the turnover of 5-HT in both the nucleus accumbens and the striatum showed an inverse relationship with the 5-HT levels and this may indicate saturation of MAO by the monoamine, in areas of higher concentration No such relationship was evident for the turnover of DA

It is apparent therefore that the distribution of the monoamines DA, NA and 5-HT is heterogeneous in both the nucleus accumbens and striatum However within each of these areas a significant degree of overlap between the monoamines was observed and interactions may therefore be possible

The nucleus accumbens has been reported to be innervated by noradrenergic neurons originating from both the LC via the dorsal noradrenergic bundle and from the subcoeruleus

nuclei via the ventral noradrenergic bundle (Section 1 2 1) However the exact source and distribution of the noradrenergic input to the nucleus accumbens has not been established Noradrenergic modulation of mesolimbic DA function has been suggested to be important in the mechanism of action of antidepressant drugs such as desipramine (Section 2 3)

In rats receiving direct 6-OHDA lesions of the LC, the greater extent of depletion of NA in the frontal cortex and hippocampus compared with the hypothalamus provided evidence that LC neurons had been lesioned MFB lesions resulted in marked reductions in NA concentration in the frontal cortex and hypothalamus which demonstrated that neurons arising in the subcoeruleus nuclei of the pons medulla had been lesioned (Section 5 3 1)

DSP4 treatment (50mg/kg i p 10 days previously) and direct LC lesion by local infusion of 6-OHDA resulted in significant decreases in NA content in the rostral nucleus accumbens, both dorsally and ventrally The medial and caudal areas were not affected These results therefore confirm reports of LC innervation of the nucleus accumbens and furthermore provide evidence that the LC neurons project to the rostral part of the nucleus accumbens DA and 5-HT concentrations were not found to be affected by DSP4 treatment or LC lesions

DSP4 lesions were observed to cause increases in HVA/DOPAC ratios in the ventromedial and ventrocaudal areas of the nucleus accumbens, suggesting a greater COMT activity in these areas These findings suggest an increase in COMT activity in these areas of the nucleus accumbens following noradrenergic denervation and a possible shift to extraneuronal metabolism of DOPAC by COMT The increased DOPAC turnover may be more evident in the medial and caudal areas of the nucleus accumbens because of the greater number of DA terminals located in these areas The proliferation of dopaminergic terminals to compensate for degenerated noradrenergic terminals following chronic NA denervation may also explain this increase in turnover

6-OHDA lesions of the MFB resulted in depletion of NA in all areas of the nucleus accumbens,

demonstrating a more widespread innervation of the nucleus accumbens by neurons of the subcoeruleus nuclei of the pons medulla DA and HVA concentrations were also significantly decreased in all areas of the nucleus accumbens following MFB lesions. ... DOPAC levels were significantly reduced in the ventrorostral area These results are in support of reports that DA neurons from the substantia nigra and VTA form part of the MFB The DOPAC/DA and HVA/DA ratios were found to be significantly increased in the medial and caudal parts of the nucleus accumbens, while the HVA/DA and HVA/DOPAC ratios were increased in the ventrorostral area of the nucleus accumbens after MFB lesions These findings imply that there is an increase in MAO activity in the medial and caudal areas which are innervated by subcoeruleus NA neurons only, while COMT activity appears to be increased in the ventrorostral nucleus accumbens after MFB lesioning It is possible that these changes could be due to different compensatory increases in the activity of the dopaminergic system resulting from the different influence of LC and subcoeruleus NA neurons Also, DA concentrations were observed to be significantly higher in the medial and caudal areas of the nucleus accumbens than in the rostral area (Section 4 3 1), and after MFB lesioning a greater number of DA neurons could remain intact in these areas This would allow more intraneuronal formation of DOPAC by MAO than in the rostral areas In support of this suggestion the DOPAC concentration was found to be significantly lower in the ventrorostral area than in any other area of the nucleus accumbens It is also possible that injury of DA terminals with the resulting loss of DA uptake sites shifts the route of inactivation from reuptake and subsequent metabolism to DOPAC, to extraneuronal conversion by COMT An additional explanation could be due to the extremely sparse DA innervation of the ventrorostral area of the nucleus accumbens The DA content and number of DA terminals in the ventrorostral area of the nucleus accumbens (Sections 4 3 1 and 7 2 2 2) was found to be significantly lower than in any other area of the nucleus accumbens After MFB lesions the DA level was decreased to almost undetectable levels (Section 5 3 4) suggesting almost complete dopaminergic denervation Metabolism of DA may therefore occur mainly extraneuronally by the action of COMT

resulting in the increased HVA/DOPAC ratio that was observed. There also remains the possibility that the increase in DA metabolism may also be a result of noradrenergic denervation in a system where noradrenergic activity may have an inhibitory effect on the dopaminergic system.

MFB lesions resulted in a significant decrease in 5-HT levels in the ventrocaudal area of the nucleus accumbens, which corresponds to the area of highest 5-HT concentration (Section 4.3.1). This decrease may indicate loss of selectivity of 6-OHDA at the concentration used in this study, but may also reflect changes secondary to the NA and DA lesions. The 5-HIAA/5-HT ratio was observed to be increased in the medial and caudal areas of the nucleus accumbens, as was found with the DOPAC/DA ratio. This agrees with the suggestion that MAO activity is increased in these areas of the nucleus accumbens after lesions of the MFB. The increased 5-HT turnover could be due to a compensatory mechanism following depletion of 5-HT, however 5-HT was significantly decreased only in the ventrocaudal area of the nucleus accumbens whilst the increase in turnover was observed in both medial and caudal areas. It is possible that the increased 5-HT turnover may be due to indirect effects of noradrenergic denervation. It has been reported that NA released from noradrenergic terminals in the median raphe inhibits the activity of 5-HT neurons and therefore the increased 5-HT turnover in the nucleus accumbens observed after MFB lesions in this study may also be a result of noradrenergic denervation.

The distribution of DA D1 and D2 receptors in the nucleus accumbens was investigated and to this end optimal binding conditions for these receptors in rat nucleus accumbens were determined. For DA D1 receptors the radioligand, [^{3}H]SCH23390 was used and for DA D2 receptors [^{3}H]spiperone was used. The specific binding of [^{3}H]SCH23390 and [^{3}H]spiperone was found to be dependent on incubation time, buffer pH and protein concentration. The specific binding of [^{3}H]SCH23390 and [^{3}H]spiperone was optimal in 50mM Tris HCl buffer at pH 7.4 (at 25^{0}C) containing 120mM NaCl, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, 0.1% ascorbic

acid and 1μ M pargyline. Equilibrium was reached after 30 minutes for [^{3}H]SCH23390 and after 15 minutes for [^{3}H]spiperone when incubated at 37°C. The specific binding of [^{3}H]SCH23390 showed a linear relationship with protein concentrations up to 3.0mg/ml whereas the specific binding of [^{3}H]spiperone increased linearly only up to protein concentrations of 0.18mg/ml. Cis-flupenthixol (1μ M) was shown to be a selective displacer for nonspecific binding of [^{3}H]SCH23390 and this ligand was also displaced with high affinity by butaclamol but not by the DA D2 selective drug, sulpiride. Sulpiride (10μ M) was demonstrated to be a selective displacer for nonspecific binding of [^{3}H]s one. Drugs active at both DA D1 and D2 receptors, butaclamol and cis-flupenthixol, also displaced [^{3}H]spiperone with high affinity, and there was evidence that the 5-HT_2 antagonist, ketanserin also displaced this ligand. Under the present conditions, therefore, [^{3}H]SCH23390 and [^{3}H]spiperone have been considered to be suitable ligands for the investigation of DA D1 and D2 receptors in rat nucleus accumbens.

The presence of both DA D1 and D2 receptors in the nucleus accumbens was demonstrated in this investigation. The DA D1 receptor density was found to be 3-9 times higher than the DA D2 receptor density in this nucleus. The present study also provides evidence for the heterogeneity of distribution of both DA D1 and D2 receptors within the nucleus accumbens of the rat. DA D1 receptor density was found to roughly follow the dopaminergic innervation, being low in the rostral and high in the medial and caudal areas. However, there were no dorsoventral differences observed in DA D1 receptor density in any coronal section of the nucleus accumbens whereas in the rostral nucleus accumbens the DA uptake and content were 2 to 2.5 times lower ventrally than dorsally. It therefore appears that although the ventrorostral area of the nucleus accumbens is more sparsely innervated by DA neurons than the dorsorostral area, similar densities of postsynaptic DA D1 receptors are present. DA turnover was shown to be significantly higher in the ventrorostral area of the nucleus accumbens than in the dorsorostral area (Section 4.3.2) and this suggests that although there are fewer DA terminals in the ventrorostral area, these neurons are more active than in the

dorsorostral area, and can therefore activate a similar number of postsynaptic DA D1 receptors

In contrast to DA D1 receptors, the DA D2 receptor distribution in the nucleus accumbens does not follow DA terminal distribution as measured by either content (Section 4.3.1) or uptake (Section 7.3.3) The DA D2 receptor density was significantly lower in the ventrorostral and higher in the dorsomedial areas than in any other area of the nucleus accumbens. There was no difference in DA D2 receptor density in medial and lateral parts of the dorsomedial area, thus localizing the highest DA D2 receptor density in the nucleus accumbens to this area. The DA D2 receptor density in the dorsorostral, ventromedial, dorsocaudal and ventrocaudal areas was found to be similar This was not the case for the DA content. The DA concentration was found to be 2.4 to 3.5 times lower in the dorsorostral nucleus accumbens than in the ventromedial, dorsocaudal, and ventrocaudal areas. DA uptake into dorsorostral nucleus accumbens slices was also significantly lower than DA uptake into ventromedial and dorsomedial slices. In the ventrorostral area of the nucleus accumbens where DA content and innervation were half of that observed in the dorsorostral area, DA D2 receptors were barely detectable. This would imply that there are DA terminals in the ventrorostral area of the nucleus accumbens and that they have considerably fewer inhibitory DA D2 autoreceptors than DA terminals in the dorsorostral, medial and caudal areas. This could account for the greater activity of the DA terminals in the ventrorostral area. The absence of DA autoreceptor-mediated feedback inhibition of DA release from these terminals could give rise to greater DA release, depletion of DA stores and the relatively greater DA turnover observed in this area. In agreement with there being very few DA D2 receptors in the ventrorostral area of the nucleus accumbens, no significant DA D2 receptor-mediated inhibition of the K^+-stimulated release of [^{3}H]DA or [^{14}C]ACh was observed in this area. A marked difference between DA D2 receptor density and DA innervation was also observed in the dorsomedial area of the nucleus accumbens. Twice as many DA D2 receptors were present in the dorsomedial than in the ventromedial and dorsorostral areas, whereas the proportion of DA and cholinergic terminals was similar in

these areas. Therefore the increased number of DA D2 receptors in this area is not due to the presence of a greater number of terminals However, there could be more DA D2 autoreceptors on dopaminergic terminals or DA D2 receptors may be situated postsynaptically on cholinergic terminals. DA turnover was not found to be lower in the dorsomedial area (Section 4.3.2) which would show increased DA autoreceptor function in this area, and therefore the former explanation does not seem likely. Evidence for more DA cholinergic neurons was obtained from the [^{14}C]ACh release data as the DA D2 receptor mediated inhibition of the K^+-stimulated release of [^{14}C]ACh from dorsomedial nucleus accumbens slices was 1 7 times greater than in the dorsorostral and ventromedial areas. This finding also agrees with there being 1.9 times as many DA D2 receptors in the dorsomedial area.

There is evidence, therefore, that in the nucleus accumbens there are a greater number of DA D1 than DA D2 receptors. The distribution of these DA receptor subtypes does not appear to be superimposable although some overlap was observed DA D1 receptors are more evenly distributed and in general appear to follow the distribution of DA innervation in the nucleus accumbens whereas the DA D2 receptors do not

Antidepressants have been reported to affect noradrenergic and serotonergic systems, and there has also been evidence that chronic treatment with antidepressants leads to an increase in dopaminergic transmission, particularly in the mesolimbic system (Section 2 3). There have been conflicting reports on the effects of antidepressant treatment on DA receptors. In this study, no significant difference in DA D1 receptor density or affinity was observed in the nucleus accumbens of controls and rats treated with desipramine for either 14 or 28 days. It has been proposed that the apparent discrepancy between the findings of this study and a recent report showing decreased DA D1 receptor density after treatment with antidepressant drugs could be due to differences in the binding conditions, for example the inclusion of NaCl and the exclusion of the Ca^{++} chelator, EGTA, in the incubation buffer. Where different binding conditions are employed, the predominance of one affinity state over another may be labelled

and this could lead to different results being obtained if one affinity state is affected by antidepressant treatment more than another The twice daily intraperitoneal injection has also been suggested to provide a possible explanation for the differences observed as these could have resulted in stress. These authors also reported on the limbic system as including the olfactory tubercle, preoptic area, septum, and amygdala as well as the nucleus accumbens whereas in the present study only the nucleus accumbens tissue was included. It therefore appears that the antidepressant-induced increase in dopaminergic activity observed in behavioural studies is not due to a direct effect on either DA D1 or D2 receptors. However, changes could have occurred in second messenger systems It is also possible that the increased dopaminergic transmission evident following chronic antidepressant treatment may be an indirect consequence of antidepressant interactions with noradrenergic or serotonergic systems in the brain

This study has therefore provided evidence for a heterogeneous distribution of monoamines in the nucleus accumbens and striatum of the rat The apparent overlap between monoaminergic terminals in the different areas of the nucleus accumbens would allow interactions between these neurotransmitter systems to be possible The activity of dopaminergic neurons and the distribution of DA D1 and D2 receptors in the nucleus accumbens has also been shown to be heterogeneous and therefore future investigations of the nucleus accumbens should take these findings into account.

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APPENDIX A

CHEMICAL	SUPPLIER
[N-methyl-^{3}H]SCH23390 (80Ci/mmol) in ethanol	Amersham International
[2,3(n)-^{3}H]butyl,[4-^{3}H]Spiperone (85Ci/mmol) in toluene ethanol 1:1	Amersham International
[^{125}I]-4-Iodospiperone	N.E.N Research Products
Cis-flupenthixol	gift from H Lundbeck
Sulpiride	Sigma
Butaclamol	Ayerst
Desipramine	gift from Ciba Geigy
Pargyline	Sigma
Ascorbic acid	Merck
Ketanserin	gift from Janssen Pharmaceutica
Brij	Technicon Chemicals SA
Ascorbic acid oxidase	Boehringer Mannheim Biochemica
DSP4	gift from A ra
6-OHDA	Sigma
Inovar Vet (0 4mg/ml fentanyl and 20mg/ml droperidol)	Janssen Pharmaceutica

APPENDIX B

INSTRUMENTS

Beckman LS 9800 liquid scintillation spectrometer

Beckman LS 1801 liquid scintillation spectrometer

Beckman PHI 71 pH meter

Beckman J2-21 refrigerated centrifuge

Heidolph stirrer

Filtration unit, 20 well, (custom made)

Gilford spectrophotometer (Model STASAR)

McIlwain tissue chopper

Mettler HL 52 balance

Sartorius 1219 MP top loading balance

Sartorius 4501 microbalance

Bioanalytical system LC-304 HPLC linked to a RYT recorder and a

Varian 4270 dual channel integrator

CW Excell II micro computer

MSE Sonicator

Sorvall RC-5B refrigerated superspeed centrifuge

LKB Wallace 1260 mutigamma gamma counter

APPENDIX C

C.1 Tukey's studentized range test on data in Table 4.3

NA

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE:
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=68 MSE=2517.66
CRITICAL VALUE OF STUDENTIZED RANGE=5.128
MINIMUM SIGNIFICANT DIFFERENCE=115.07

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AREA
A A	261.50	5	ventromedial, slice 4
B A	213.32	5	ventrolateral, slice 5
B C B C	127.46	5	ventromedial, slice 3
B C C	103.18	5	ventrolateral, slice 4
C C	87.98	5	ventrolateral, slice 1
C C	69.68	5	ventrolateral, slice 2
C C	64.92	5	ventromedial, slice 2
C C	63.04	5	ventrolateral, slice 3
C C	53.94	5	dorsomedial, slice 4
C C	49.92	5	dorsolateral, slice 5
C C	44.00	5	dorsolateral, slice 3
C C	43.78	5	dorsolateral, slice 4
C C	39.84	5	dorsomedial, slice 2
C C	35.42	5	dorsomedial, slice 3
C C	33.56	5	dorsolateral, slice 2
C C	31.68	5	dorsolateral, slice 1
C C	29.98	5	ventromedial, slice 1
C	29.86	5	dorsomedial, slice 1

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: DA
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=60 MSE=2925785
CRITICAL VALUE OF STUDENTIZED RANGE=5.128
MINIMUM SIGNIFICANT DIFFERENCE=3922.7

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AREA
A	11037	5	dorsomedial slice 1
B A	10244	5	ventrolateral slice 3
B A	9565	5	dorsolateral slice 2
B A	9463	5	dorsolateral slice 1
B A	9278	5	dorsomedial slice 2
B A	9257	5	ventromedial slice 2
B A	8722	5	ventrolateral slice 2
B A C	8451	5	ventromedial slice 1
B A C	8293	5	dorsolateral slice 3
B A C	7398	5	ventrolateral slice 4
B D C	6946	5	ventrolateral slice 1
B D C	6837	5	ventromedial slice 3
B D C	6804	5	dorsomedial slice 3
B D C	6635	5	dorsolateral slice 4
D C	4585	5	dorsolateral slice 5
D E	4165	5	ventrolateral slice 5
D E	3718	5	dorsomedial slice 4
E	1598	5	ventromedial slice 4

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: DOPAC
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=68 MSE=114160
CRITICAL VALUE OF STUDENTIZED RANGE=5.128
MINIMUM SIGNIFICANT DIFFERENCE=774.85

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AREA
A	3158.2	5	ventromedial slice 1
A B	2556.5	5	dorsomedial slice 1
A B	2498.7	5	ventromedial slice 2
B C	2358.7	5	ventrolateral slice 1
B C D	2230.1	5	dorsomedial slice 2
B C D	2191.9		entrolateral slice 3
B C D	2179.2	5	ventrolateral slice 2
B E C D	2081.6	5	ventromedial slice 3
F B E C D	1921.3	5	dorsolateral slice 1
F B E C D	1850.6	5	dorsomedial slice 3
F B E C D	1835.1	5	dorsolateral slice 2
F B E C D	1824.1	5	ventrolateral slice 4
F B E C D	1799.8	5	dorsolateral slice 3
F E C D	1700.6	5	dorsolateral slice 4
F E D	1516.5	5	ventrolateral slice 5
F E G	1338.4	5	dorsolateral slice 5
F G	1300.9	5	dorsomedial slice 4
G	692.1	5	ventromedial slice 4

HVA

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: HVA
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE, BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=68 MSE=9214.87
CRITICAL VALUE OF STUDENTIZED RANGE=5.128
MINIMUM SIGNIFICANT DIFFERENCE=220.14

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AREA
A	989.12	5	ventrolateral slice 3
A			
B A	966.38	5	ventrolateral slice 2
B A			
B A C	800.42	5	ventromedial slice 2
B A C			
B A C	788.00	5	ventromedial slice 1
B C			
B D C	741.64	5	dorsolateral slice 2
B D C			
B D C	727.44	5	ventrolateral slice 1
B D C			
B D C	712.96	5	dorsolateral slice 3
B D C			
B D C	703.94	5	ventrolateral slice 4
B D C			
B D C	678.18	5	dorsolateral slice 1
B D C			
B D C	662.52	5	ventromedial slice 3
D C			
E D C	634.76	5	dorsomedial slice 1
E D C			
E F D C	604.16	5	dorsolateral slice 4
E F D C			
E F D C	594.58	5	dorsomedial slice 2
E F D			
E F D	523.88	5	dorsomedial slice 3
E F			
E F G	438.70	5	ventrolateral slice 5
E F G			
E F G	416.48	5	dorsolateral slice 5
F G			
F G	387.40	5	dorsomedial slice 4
G			
G	298.18	5	ventromedial slice 4

5-HT

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: 5-HT
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=68 MSE=8640.77
CRITICAL VALUE OF STUDENTIZED RANGE=5.128
MINIMUM SIGNIFICANT DIFFERENCE=213.18

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AREA
A A	767.74	5	ventromedial slice 4
A A	750.62	5	ventromedial slice 3
B A A	734.08	5	ventrolateral slice 4
B A A	696.98	5	ventrolateral slice 5
B A C C	579.32	5	ventrolateral slice 3
B D C C	523.76	5	dorsolateral slice 5
D C C	432.52	5	ventrolateral slice 2
E D C C	469.74	5	ventromedial slice 2
E F D C C	409.06	5	dorsomedial slice 3
G E F D C C	400.66	5	dorsomedial slice 4
G E F D C C	393.54	5	dorsolateral slice 4
G E F D C C H	367.90	5	dorsolateral slice 3
G E F D H	312.22	5	ventrolateral slice 1
G E F H	263.66	5	dorsomedial slice 2
G F H	242.38	5	dorsolateral slice 2
G F H	240.86	5	ventromedial slice 1
G H	193.38	5	dorsolateral slice 1
H	167.16	5	dorsomedial slice 1

5-HIAA

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: 5-HIAA
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE.
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=68 MSE=5619.18
CRITICAL VALUE OF STUDENTIZED RANGE=5.128
MINIMUM SIGNIFICANT DIFFERENCE=171.91

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AREA
A	989.12	5	ventromedial slice 4
A			
B A	837.52	5	ventrolateral slice 5
B			
B	785.92	5	ventromedial slice 3
B			
B	781.74	5	ventrolateral slice 4
B C			
B C	669.84	5	ventrolateral slice 3
C			
D C	594.28	5	dorsolateral slice 5
D C			
D C E	511.88	5	ventrolateral slice 2
D C E			
D C E	503.90	5	dorsomedial slice 4
D C E			
D C E	501.64	5	dorsolateral slice 4
D C E			
D C E	500.42	5	ventromedial slice 2
D E			
D F E	473.38	5	dorsolateral slice 3
F E			
F E	417.84	5	dorsomedial slice 3
F E			
F E	407.60	5	ventrolateral slice 1
F E			
F E	370.42	5	ventromedial slice 1
F E			
F E	351.88	5	dorsolateral slice 2
F			
F	326.58	5	dorsomedial slice 2
F			
F	303.52	5	dorsolateral slice 1
F			
F	302.66	5	dorsomedial slice 1

C.2 Tukey's studentized range test on data in Table 7.1

DA D1 Receptor Bmax

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: LOGBMAX
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=25 MSE=.0623392
CRITICAL VALUE OF STUDENTIZED RANGE=4.358
MINIMUM SIGNIFICANT DIFFERENCE=.44425

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AREA
A	6.5128	6	dorsocaudal
A			
A	6.4582	6	ventrocaudal
A			
A	6.3692	6	dorsomedial
A			
A	6.1949	6	ventromedial
B	4.8666	6	ventrorostral
B			
B	4.7691	6	dorsorostral

DA D1 Receptor Kd

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: LOGKD
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=25 MSE=.0783903
CRITICAL VALUE OF STUDENTIZED RANGE=4.358
MINIMUM SIGNIFICANT DIFFERENCE=.49817

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AREA
A	-0.9415	6	ventrocaudal
A A	-0.9872	6	dorsomedial
A A	-1.0350	6	ventromedial
A A	-1.0619	6	dorsocaudal
A	-1.2740	6	ventrorostral
B	-2.1765	6	dorsorostral

DA D2 Receptor Bmax

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: BMAX
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE, BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=26 MSE=239.223
CRITICAL VALUE OF STUDENTIZED RANGE=4.345
MINIMUM SIGNIFICANT DIFFERENCE=29.422

WARNING: CELL SIZES ARE NOT EQUAL.
HARMONIC MEAN OF CELL SIZES=5.21739

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AREA
A	195.817	6	dorsomedial
B	116.300	6	ventrocaudal
B B	104.100	5	ventromedial
B B	102.750	6	dorsocaudal
B B	101.125	4	dorsorostral
C	15.280	5	ventrorostral

DA D2 Receptor KD

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: KD
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE, BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=20 MSE=1.3E-04
CRITICAL VALUE OF STUDENTIZED RANGE=4.445
MINIMUM SIGNIFICANT DIFFERENCE=.02291

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AREA
A	0.055200	5	ventrocaudal
A			
A	0.053800	5	dorsorostral
A			
A	0.052800	5	dorsomedial
A			
A	0.051200	5	dorsocaudal
A			
B A	0.038800	5	ventromedial
B			
B	0.023800	5	ventrorostral

C.3 Ryan-Einot-Gabriel-Welsch test on data in Table 7.3

^{3}H DA release

GENERAL LINEAR MODELS PROCEDURE

RYAN-EINOT-GABRIEL-WELSCH MULTIPLE F TEST FOR VARIABLE: DA release
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE

ALPHA=0.05 DF=31 MSE=2.7E-04

NUMBER OF MEANS	2	3
CRITICAL F	4.159732	3.304882

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

REGWF	GROUPING	MEAN	N	AREA
	A	0.153708	12	rostral
	A A			
	A	0.151467	12	medial
	A A			
	A	0.141508	12	caudal

^{14}C ACh release

GENERAL LINEAR MODELS PROCEDURE

RYAN-EINOT-GABRIEL-WELCH MULTIPLE F TEST FOR VARIABLE: ACH release
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE

ALPHA=0.05 DF=31 MSE=1.8E-04

NUMBER OF MEANS	2	3
CRITICAL F	4.15862	3.30482

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

REGWF GROUPING	MEAN	N	A REA
A	0.214775	12	caudal
B	0.101417	12	medial
C	0.111733	12	rostral

Tukey's studentized range test on data in Table 7.3

^{14}C choline uptake

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: ^{3}H choline uptake
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE.
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=6 MSE=2704.92
CRITICAL VALUE OF STUDENTIZED RANGE=4.339
MINIMUM SIGNIFICANT DIFFERENCE=112.84

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	SL
A	548.00	4	caudal
B	389.75	4	medial
B			
B	359.50	4	rostral

C.4 Tukey's studentized range test on data in Table 7.4

^{3}H DA uptake

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: DA uptake
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=12 MSE=.0539091
CRITICAL VALUE OF STUDENTIZED RANGE=4.199
MINIMUM SIGNIFICANT DIFFERENCE=.43633

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AR
A	2.0814	5	ventromedial
A			
A	2.0426	5	dorsomedial
A			
A	1.8629	5	dorsorostral
B	1.0202	5	ventrorostral

^{14}C choline uptake

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: choline uptake
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=12 MSE=.0211944
CRITICAL VALUE OF STUDENTIZED RANGE=4.199
MINIMUM SIGNIFICANT DIFFERENCE=.27338

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT

TUKEY GROUPING	MEAN	N	AR
A	5.92497	5	ventromedial
A			
A	5.87758	5	dorsomedial
B	5.60315	5	dorsorostral
C	5.31455	5	ventrorostral

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: DA
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=27 MSE=4.3E-04
CRITICAL VALUE OF STUDENTIZED RANGE=3.870
MINIMUM SIGNIFICANT DIFFERENCE=.02542

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT

TUKEY GROUPING	MEAN	N	AREA
A	0.016280	10	ventrorostral
B	0.043030	10	ventromedial
B			
B	0.050730	10	dorsorostral
B			
B	0.052990	10	dorsomedial

^{14}C ACh release SULPIRIDE - QUINPIROLE

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: ACH
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE, BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=27 MSE=4.4E-04
CRITICAL VALUE OF STUDENTIZED RANGE=3.870
MINIMUM SIGNIFICANT DIFFERENCE= 02579

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT

TUKEY GROUPING	MEAN	N	AREA
A	0.009020	10	ventrorostral
B	0 036990	10	ventromedial
B	0 038720	10	dorsorostral
C	0.064630	10	dorsomedial

C.5 Tukey's studentized range test on data in Table 7.5

^{3}H DA release, AREA * TREATMENT interaction

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: DA_REL
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=104 MSE=3.7E-04
CRITICAL VALUE OF STUDENTIZED RANGE=4.106
MINIMUM SIGNIFICANT DIFFERENCE=.01765

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AR_TR
A	0.186425	20	dorsorostral + dorsomedial, sulpiride
B	0.161050	20	dorsorostral + dorsomedial, nil
C	0.142245	20	ventrorostral + ventromedial, sulpiride
C C	0.134565	20	dorsorostral + dorsomedial, quinpirole
C C	0.133600	20	ventrorostral + ventromedial, nil
D	0.112590	20	ventrorostral + ventromeidal, quinpirole

^{14}C ACh release AREA * TREATMENT interaction

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: ACH_REL
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=101 MSE=3.0E-04
CRITICAL VALUE OF STUDENTIZED RANGE=4.108
MINIMUM SIGNIFICANT DIFFERENCE=.01588

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	AR_TR
A	0.187690	20	dorsorostral + dorsomedial, sulpiride
A			
B A	0.179870	20	dorsorostral + dorsomedial, nil
B			
B C	0.164215	20	ventrorostral + ventromedial, sulpiride
C			
C	0.163809	20	ventrorostral + ventromedial, nil
D	0.141210	20	ventrorostral + ventromedial, quinpirole
D			
D	6015	20	dorsorostral + dorsomedial, quinpirole

^{14}C ACh release, SLICE * TREATMENT interaction

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: ACH_REL
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=101 MSE=3.0E-04
CRITICAL VALUE OF STUDENTIZED RANGE=4.108
MINIMUM SIGNIFICANT DIFFERENCE=.01588

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	SL_TR
A	0.232955	20	ventromedial + dorsomedial, sulpiride
A			
A	0.227635	20	ventromedial + dorsomedial, nil
B	0.182145	20	ventromedial + dorsomedial, quinpirole
C	0.118950	20	ventrorostral + dorsorostral, sulpiride
C			
C	0.116035	20	ventrorostral + dorsorostral, nil
D	0.095080	20	ventrorostral + dorsorostral, quinpirole

^{14}C ACh release SLICE * AREA interaction

GENERAL LINEAR MODELS PROCEDURE

TUKEY'S STUDENTIZED RANGE (HSD) TEST FOR VARIABLE: ACH_REL
NOTE: THIS TEST CONTROLS THE TYPE I EXPERIMENTWISE ERROR RATE,
BUT GENERALLY HAS A HIGHER TYPE II ERROR RATE THAN REGWQ

ALPHA=0.05 DF=101 MSE=3.0E-04
CRITICAL VALUE OF STUDENTIZED RANGE=3.694
MINIMUM SIGNIFICANT DIFFERENCE=.01186

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

TUKEY GROUPING	MEAN	N	SL_AR
A	0.215347	30	dorsomedial (nil + quinpirole + sulpiride)
A			
A	0.213143	30	ventromedial (nil + quinpirole + sulpiride)
B	0.120370	30	dorsorostral (nil + quinpirole + sulpiride)
C	0.099673	30	ventrorostral (nil + quinpirole + sulpride)